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# Flow-independent nitric oxide exchange parameters in healthy adults

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**Shin, Hye-Won, Christine M. Rose-Gottron, Federico Perez, Dan M. Cooper, Archie F. Wilson, and Steven C. George.** Flow-independent nitric oxide exchange parameters in healthy adults. *J Appl Physiol* 91: 2173–2181, 2001.—Currently accepted techniques utilize the plateau concentration of nitric oxide (NO) at a constant exhalation flow rate to characterize NO exchange, which cannot sufficiently distinguish airway and alveolar sources. Using nonlinear least squares regression and a two-compartment model, we recently described a new technique (Tsoukias et al. *J Appl Physiol* 91: 477–487, 2001), which utilizes a preexpiratory breath hold followed by a decreasing flow rate maneuver, to estimate three flow-independent NO parameters: maximum flux of NO from the airways ( $J_{NO,max}$ , pl/s), diffusing capacity of NO in the airways ( $D_{NO,air}$ ,  $pl \cdot s^{-1} \cdot ppb^{-1}$ ), and steady-state alveolar concentration ( $C_{alv,ss}$ , ppb). In healthy adults ( $n = 10$ ), the optimal breath-hold time was 20 s, and the mean (95% intramaneuver, intrasubject, and intrapopulation confidence interval)  $J_{NO,max}$ ,  $D_{NO,air}$ , and  $C_{alv,ss}$  are 640 (26, 20, and 15%) pl/s, 4.2 (168, 87, and 37%)  $pl \cdot s^{-1} \cdot ppb^{-1}$ , and 2.5 (81, 59, and 21%) ppb, respectively.  $J_{NO,max}$  can be estimated with the greatest certainty, and the variability of all the parameters within the population of healthy adults is significant. There is no correlation between the flow-independent NO parameters and forced vital capacity or the ratio of forced expiratory volume in 1 s to forced vital capacity. With the use of these parameters, the two-compartment model can accurately predict experimentally measured plateau NO concentrations at a constant flow rate. We conclude that this new technique is simple to perform and can simultaneously characterize airway and alveolar NO exchange in healthy adults with the use of a single breathing maneuver.

diffusing capacity; airways; alveolar

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EXHALED NITRIC OXIDE (NO) arises from the airways and alveoli in human lungs and continues to hold promise as a noninvasive marker of airway inflammation (4, 7, 22, 26, 28, 32, 35). However, reported exhaled NO concentrations vary widely in healthy and diseased populations (3, 10, 12, 13, 19, 21, 24, 27, 31). The variability can be attributed, in part, to differences in

the technique, the origin of the exhaled NO (airway or alveolar source), and the presence of an inflammatory disease (11, 16, 18, 27).

The American Thoracic Society (ATS) and the European Respiratory Society recently recommended a constant exhalation flow rate ( $\sim 50$  and  $\sim 250$  ml/s, respectively) breathing maneuver as the standardized procedure for collection of NO (15, 30). This technique utilizes the plateau concentration ( $C_{NO,plat}$ ) to characterize NO metabolism or exchange. Although  $C_{NO,plat}$  at an exhalation flow rate of 50 and 250 ml/s is predominantly from the airway and alveolar compartments, respectively,  $C_{NO,plat}$  alone cannot fully characterize NO exchange in the airway and alveolar regions of the lungs. Therefore, we recently described a new technique (34), which utilizes a preexpiratory breath hold followed by a decreasing flow rate maneuver, to separately characterize airway and alveolar NO exchange dynamics. Characterization of the airways consists of two parameters: maximum flux of NO from the airways ( $J_{NO,max}$ , pl/s) and the diffusing capacity of NO in the airways ( $D_{NO,air}$ ,  $pl \cdot s^{-1} \cdot ppb^{-1}$ ). Characterization of alveolar exchange is accomplished using the steady-state alveolar concentration ( $C_{alv,ss}$ , ppb). The preexpiratory breath hold and decreasing flow rate exhalation sample a large range of gas bolus airway compartment residence times, which are necessary for characterization of all three parameters (34). Thus the technique takes advantage of the flow dependence of exhaled NO concentration to simultaneously estimate three flow-independent parameters. We hypothesize that the flow-independent parameters not only provide greater specificity for NO exchange dynamics but also can be used to accurately predict exhaled NO concentration at a constant flow rate.

Our initial description (34) focused on characterizing the intrinsic variance (intramaneuver) of the technique in estimating the parameters, i.e., the contribution to the variance in the parameter estimates due to limitations in the analytic instrumentation and the two-

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compartment model. However, variability within a subject (intrasubject) and within a population (intrapopulation) needs further characterization before the technique might be used as a clinical tool. Thus the aims of this study are fivefold: 1) to determine average values for the flow-independent parameters in a healthy population of adults without lung disease, 2) to characterize the intrasubject and intrapopulation variability in the flow-independent parameters, 3) to determine correlation of flow-independent parameters with standard spirometry [e.g., forced expiratory volume in 1 s ( $FEV_1$ )], 4) to determine the optimal preexpiratory breath-hold time, and 5) to demonstrate that the flow-independent parameters can be used in a two-compartment model to accurately predict exhaled NO concentration at a constant flow rate.

## METHODS

**Subjects.** Ten nonsmoking healthy adults, between 20 and 35 yr of age (6 men and 4 women), were recruited to participate in the study. Subjects were categorized as healthy on the basis of their standard spirometry [ $>80\%$  of the predicted value of forced vital capacity (FVC),  $FEV_1$ , and  $FEV_1/FVC$ ], the absence of pulmonary disease by history, and the absence of smoking and allergies by history. The Institutional Review Board at the University of California, Irvine, approved the protocol, and informed consent was obtained from all subjects before the experiments.

**Experimental protocol.** Standard spirometry (Vmax229, SensorMedics, Yorba Linda, CA) was performed in triplicate in all subjects to measure FVC and  $FEV_1$  before the exhaled NO measurements (Table 1).

Before performing a single exhalation maneuver, each subject was allowed 3–5 min of comfortable tidal breathing. The subject then performed two types of exhalation maneuvers while wearing noseclips: 1) vital capacity maneuvers in triplicate at a constant exhalation flow of  $\sim 50$  and  $\sim 250$  ml/s according to ATS and European Respiratory Society guidelines to determine  $C_{NO,plat}$  and 2) five repetitions of a maneuver consisting of an inspiration of NO-free air from the Mylar bag to total lung capacity, a preexpiratory breath hold, and a decreasing flow rate exhalation. The breath-hold time was 10, 20, 30, or 45 s, and during exhalation the expiratory flow rate progressively decreased from  $\sim 6\%$  to  $1\%$  of the vital capacity per second (34). During the breath hold, a positive pressure of  $>5$  cmH<sub>2</sub>O was maintained by the subject to

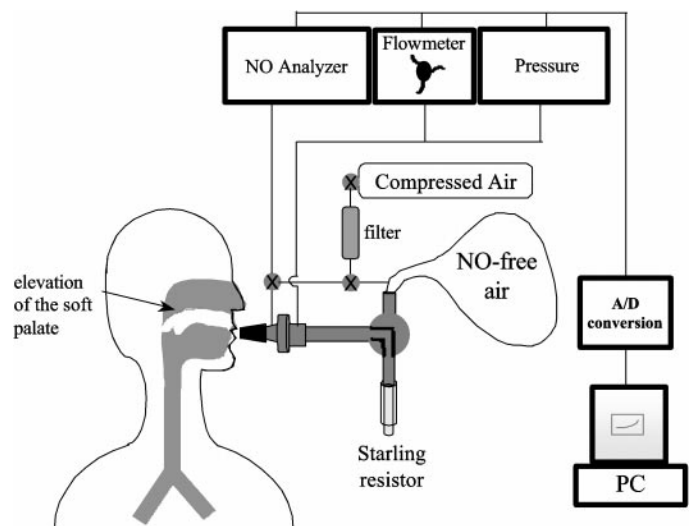


Fig. 1. Schematic of experimental setup used to collect exhalation profiles. Flow, pressure, and nitric oxide (NO) analog (A) signals are captured by the analytic instruments and converted to a digital (D) signal. A series of valves allows NO-free air to be stored in a Mylar bag for inspiration. During the breath hold, the NO analyzer samples from the NO-free air reservoir, and the subject maintains a positive pressure  $>5$  cmH<sub>2</sub>O by attempting to exhale against a closed valve. As exhalation begins, the NO analyzer samples from the exhalate, and the flow rate is manipulated by a variable Starling resistor while the expiratory effort of the subject remains constant.

prevent nasal contamination (30), and NO was sampled from an NO-free reservoir. Just before exhalation, a valve on the NO-sampling line was changed to sample from the exhaled breath, and the exhalation valve was opened, allowing the patient to expire. Control of the exhalation flow rate was facilitated via a Starling resistor (Hans Rudolph, Kansas City, MO) with a variable resistance. A schematic of the experimental apparatus is presented in Fig. 1, and details have been previously described (34).

**Airstream analysis.** A rapid-response chemiluminescence NO analyzer (model NOA280, Sievers, Boulder, CO) with a 10–90% response time of  $<200$  ms was used to measure exhaled NO concentration. The sampling flow rate was adjusted to 200 ml/min with an operating reaction cell pressure of 7.4 mmHg. The instrument was calibrated on a daily basis using a certified NO gas (45 ppm in N<sub>2</sub>, Sievers) tank and zero gas. The zero point calibration was performed with an

Table 1. Physical characteristics of subjects

Subject No.	Gender	Age, yr	Wt, lb.	$V_{air}$ , ml	FVC		$FEV_1$		$FEV_1/FVC$
					liter	%pred	liter	%pred	
1	F	25	114	139	3.46	94	3.08	100	89
2	F	33	101	134	3.41	118	2.88	115	85
3	M	35	145	180	4.60	95	3.76	93	82
4	F	31	97	128	3.00	99	2.52	95	84
5	M	26	162	188	4.22	99	3.48	98	82
6	M	22	145	167	4.71	102	4.03	101	86
7	M	29	145	174	4.06	90	3.57	94	88
8	M	35	140	175	4.40	97	3.88	103	88
9	M	21	153	174	4.77	100	4.52	110	95
10	F	20	128	148	3.34	90	3.02	90	90
Mean		28	133	161	4.00	98	3.47	100	87

FVC, forced vital capacity;  $FEV_1$ , forced expiratory volume in 1 s;  $V_{air}$ , volume of airway; F, female; M, male; %pred, percent predicted.

NO filter (Sievers) and performed immediately before the collection of a profile. The flow rate and pressure signals were measured using a pneumotachometer (model RSS100, Hans Rudolph). The pneumotachometer was also calibrated daily and set to provide the flow in units of STPD and pressure in cmH<sub>2</sub>O. The analog signals of NO, flow, and pressure were digitized using an analog-to-digital card at a rate of 50 Hz and stored for further analysis.

**Parameter estimation.** A previously described two-compartment model was used to estimate three flow-independent parameters ( $J_{NO,max}$ ,  $D_{NO,air}$ , and  $C_{alv,ss}$ ) (32, 34, 35). Figure 2 is a simple schematic of the two-compartment model and flow-independent parameters. Mathematical identification of the parameters has been previously described in detail (34), and only the salient features are presented here. Equation 1 is the governing equation for the model, which predicts the exhaled concentration ( $C_{exh}$ , ppb) as a function of the residence time ( $\tau_{res}$ ) of each differential bolus of air in the airway compartment, the volume of the airway compartment ( $V_{air}$ ), and the remaining three parameters ( $J_{NO,max}$ ,  $D_{NO,air}$ , and  $C_{alv,ss}$ )

$$C_{exh}(t) = \left( C_{alv,ss} - \frac{J_{NO,max}}{D_{NO,air}} \right) e^{-\frac{D_{NO,air}}{V_{air}} \tau_{res}(t)} + \frac{J_{NO,max}}{D_{NO,air}} \quad (1)$$

$V_{air}$  (ml) is approximated by the physiological dead space, which is estimated using the subject weight in pounds plus age in years (6, 34). The  $\tau_{res}$  is determined using a previously described (34) backward integration algorithm in which the flow rate history of the bolus is utilized.

Identification of the unknown parameters ( $J_{NO,max}$ ,  $D_{NO,air}$ , and  $C_{alv,ss}$ ) is accomplished by nonlinear least squares utilizing a conjugated direction algorithm to minimize the sum of square of the residuals ( $R_{LS}$ ) between the model's prediction and the experimental data. Figure 3 is a representative exhalation profile simulated by the model. The model does not precisely predict phases I and II of the exhalation profile, where the accumulated NO during breath holding in the conducting airways and transition region of the lungs exits the mouth. This discrepancy is attributed primarily to axial diffusion, which our model neglects. Although the precise shape of phase I cannot be accurately simulated with the model, the absolute amount of NO in phases I and II can be predicted. Thus our technique utilizes the information from phases I and II (where  $\tau_{res}$  is large and,

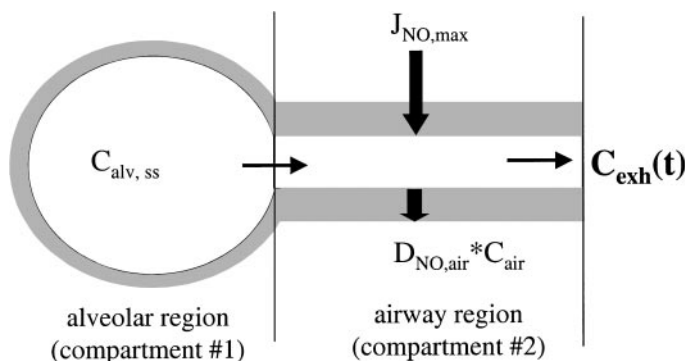


Fig. 2. Schematic of 2-compartment model used to describe NO exchange dynamics. Exhaled NO concentration ( $C_{exh}$ ) depends on 3 flow-independent parameters: maximum flux of NO from the airways ( $J_{NO,max}$ , pl/s), diffusing capacity of NO in the airways ( $D_{NO,air}$ , pl·s<sup>-1</sup>·ppb<sup>-1</sup>), and steady-state alveolar concentration ( $C_{alv,ss}$ , ppb).  $C_{air}$ , concentration of NO in the gas phase of the airway compartment.

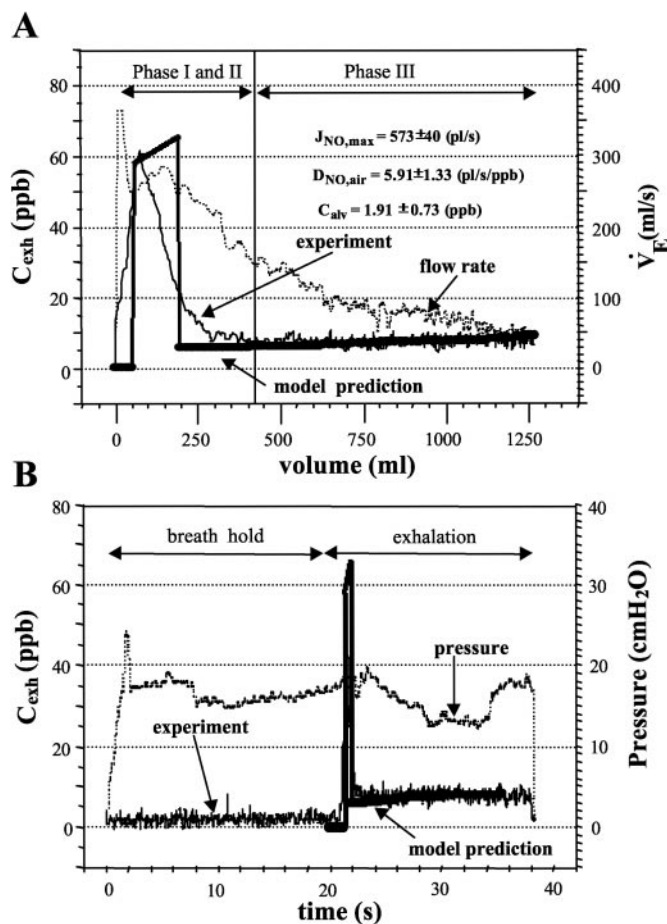


Fig. 3. Representative exhaled NO concentration profiles utilizing the 20-s preexpiratory breath hold followed by a decreasing flow rate maneuver shown as a function of exhaled volume (A) or time (B). Light solid line, exhaled NO concentration (ppb); dashed line, exhalation flow rate (A) or pressure (B); dark solid line, best-fit model prediction of the exhalation curve using nonlinear least-squares regression. Optimal values for the 3 parameters,  $J_{NO,max}$ ,  $D_{NO,air}$ , and  $C_{alv,ss}$ , are also presented. For detailed description of parameters and regression techniques see METHODS.

hence, the sensitivity to  $D_{NO,air}$  is high) by forcing the model to simulate the total amount of NO exiting in phases I and II of the exhalation in addition to simulating the precise  $C_{exh}$  over phase III. Thus the fitting of the experimental data includes a minimization of the sum of two terms: 1) the squared residual in the average concentrations in phases I and II weighted by the number of data points and 2) the sum of the squared residual of  $C_{exh}$  in phase III of the exhalation profile (34). To ensure complete emptying of the airway compartment after breath hold, we define the transition from phases II and III as the point in the exhalation for which the slope ( $dC_{exh}/dV$ , where  $V$  is volume) of the exhalation profile is zero.

An alternative presentation of the three flow-independent parameters includes the use of the mean (over radial position) tissue concentration in the airways ( $C_{tiss,air}$ ), instead of  $J_{NO,max}$  (28).  $C_{tiss,air}$  is simply the ratio  $J_{NO,max}/D_{NO,air}$ .

**Statistical analysis.** One of the aims of the present study is to characterize the variability or uncertainty in the estimate of the flow-independent parameters. If one estimates the three parameters from a single maneuver from a single subject, the variability in the estimated values of the param-

eters is due to the intrinsic variability of the technique, which includes the accuracy of the model and the analytic instrumentation (intramanuever). One can then repeat the same maneuver multiple times, and the variability in the repeated estimates is due to reproducibility of the breathing maneuver (intermanuever or intrasubject). Finally, one can repeat the same series of breathing maneuvers across a population of individuals, and the variability is due to the intrinsic variation of the population (intersubject or intrapopulation). The intramanuever variability has been described previously (34) and can be characterized by the  $100(1 - \alpha)\%$  normalized confidence interval ( $\Delta\bar{I}_{1-\alpha,i}^m$ ) using the following relationship

$$\Delta\bar{I}_{1-\alpha,i}^m = [\pm pF_{1-\alpha}(p, n-p)e_1(\lambda_1)^{-1/2}]_{i-\text{row}}/y_i \quad (2)$$

where  $y_i$  is the estimated value of parameter  $i$  (i.e.,  $J_{\text{NO,max}}$ ),  $\lambda_1$  is the smaller eigenvalue of the covariance matrix,  $e_1$  is the corresponding eigenvector (5), and  $F_{1-\alpha}$  is the  $F$  statistic test for the number of estimated parameters ( $p$ , i.e., 3 in our case) and the number of data points ( $n$ ). This estimate assumes additive zero mean and normally distributed measurement errors and errorless measured inputs.  $\Delta\bar{I}_{1-\alpha,i}^m$  is a positive function of  $R_{\text{LS}}$  and, thus, depends on the accuracy of the analytic instrument as well as the accuracy of the two-compartment model.

The normalized intrasubject (intermanuever) confidence interval is defined by using the standard deviation (SD) of the estimate of each of the parameters for the five repeated maneuvers

$$\Delta\bar{I}_{1-\alpha,i}^s = \pm \frac{\text{SD}}{\sqrt{n_m}} t_{1-\alpha}/y_i \quad (3)$$

where  $n_m$  is the number of breathing maneuvers and  $t_{1-\alpha}$  is the critical  $t$  value for  $n_m - 1$  degrees of freedom. The intrapopulation (intersubject) confidence interval ( $\Delta\bar{I}_{1-\alpha,i}^p$ ) is defined in a similar fashion using the SD of the mean parameter estimate from the 10 subjects.

## RESULTS

The population mean for each of the parameters ( $\hat{J}_{\text{NO,max}}$ ,  $\hat{D}_{\text{NO,air}}$ , and  $\hat{C}_{\text{alv,ss}}$ ) is presented at the four different breath-hold times in Fig. 4.  $\hat{J}_{\text{NO,max}}$ ,  $\hat{D}_{\text{NO,air}}$ , and  $\hat{C}_{\text{alv,ss}}$  do not depend significantly on the breath-hold time and range from 610 to 647 pl/s, from 3.2 to 4.5 pl·s<sup>-1</sup>·ppb, and from 2.5 to 2.8 ppb, respectively. In addition, mean values for  $\Delta\bar{I}_{0.95,i}^m$  do not vary significantly with breath-hold time and range from 15 to 30%, 37 to 67%, and 21 to 43%, for  $\hat{J}_{\text{NO,max}}$ ,  $\hat{D}_{\text{NO,air}}$ , and  $\hat{C}_{\text{alv,ss}}$ , respectively. Thus the variation across the population of healthy adults is approximately the same for each of the parameters. The estimated range for  $C_{\text{tiss,air}}$  and  $\Delta\bar{I}_{0.95,C_{\text{tiss,air}}}^p$  for different breath-hold times is 208–260 ppb and 39–53%, respectively, with no statistically significant dependence on breath-hold time.

The effect of breath-hold time on the population means of  $\Delta\bar{I}_{0.95,i}^m$  and  $\Delta\bar{I}_{0.95,i}^s$  for each parameter ( $J_{\text{NO,max}}$ ,  $D_{\text{NO,air}}$ , and  $C_{\text{alv,ss}}$ ) is presented in Fig. 5 for the 10 healthy subjects. The mean  $\Delta\bar{I}_{0.95,i}^m$  for  $J_{\text{NO,max}}$  gradually decreases with increasing breath-hold time. The largest change is from a 10-s to a 20-s breath hold (36–26%), but none of the changes between consecutive breath-hold times is statistically significant. For  $D_{\text{NO,air}}$ ,  $\Delta\bar{I}_{0.95,i}^m$  improves significantly from 10 to 20 s (542 to 168%) with no further improvement for breath

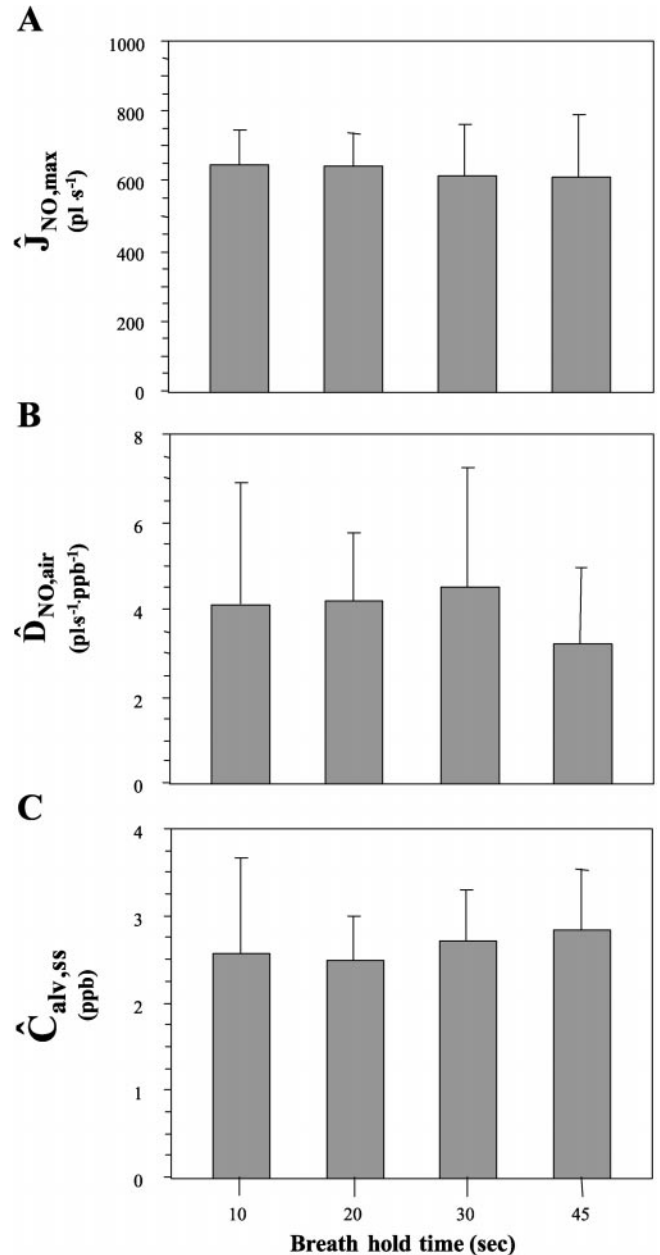


Fig. 4. Population means for the 3 flow-independent parameters at 4 different breath-hold times (10, 20, 30, and 45 s). Error bar, intrapopulation 95% confidence interval ( $\Delta\bar{I}_{0.95,i}^p$ ).

hold >20 s. Although the mean  $\Delta\bar{I}_{0.95,i}^m$  for  $C_{\text{alv,ss}}$  improves from a breath-hold time of 10–20 s (154 to 81%), this difference is not statistically significant, and  $\Delta\bar{I}_{0.95,i}^m$  remains nearly constant for breath-hold time >20 s. Mean values for  $\Delta\bar{I}_{0.95,i}^s$  for each of the parameters are not dependent on the breath-hold time and are 20, 87, and 59% for  $J_{\text{NO,max}}$ ,  $D_{\text{NO,air}}$ , and  $C_{\text{alv,ss}}$ , respectively for a 20-s breath hold.

On the basis of the above results, there is significant improvement in  $\Delta\bar{I}_{0.95,i}^m$  for  $D_{\text{NO,air}}$  and modest improvement for  $J_{\text{NO,air}}$  and  $C_{\text{alv,ss}}$  when the breath-hold time is increased from 10 to 20 s but no significant improvement when the breath-hold time is increased further. Thus Table 2 summarizes the individual data from the

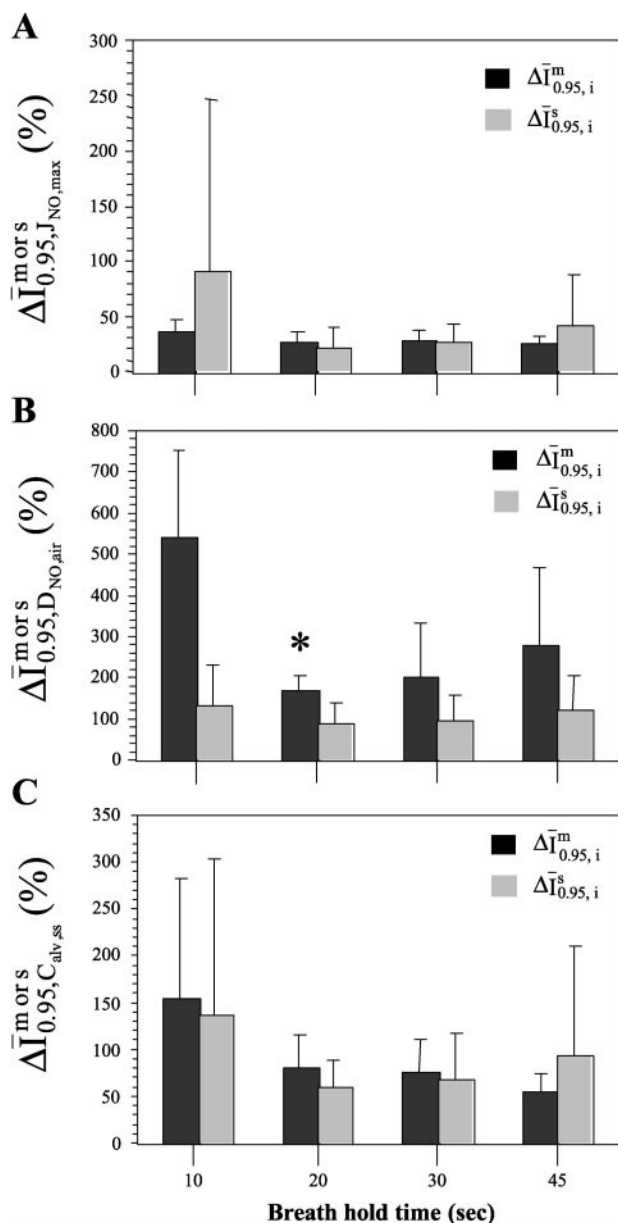


Fig. 5. Mean of  $\Delta\bar{I}_{0.95,i}^s$  and  $\Delta\bar{I}_{0.95,i}^m$  for each parameter ( $J_{NO,max}$ ,  $D_{NO,air}$ , and  $C_{alv,ss}$ ) to examine the effect of breath-hold time for 10 healthy subjects. Error bar, 95% confidence for those means. \*Statistically different from preceding breath hold ( $P < 0.05$ ).

10 subjects using a 20-s breath hold. Also included in Table 2 are the experimentally measured and model-predicted values for  $C_{NO,plat}$  with the corresponding mean experimental values for the exhalation flow rate (experimental target was 50 and 250 ml/s).

There is no correlation between estimated flow-independent parameters and standard spirometry measurements (FVC and  $FEV_1/FVC$ ) for healthy adults. In addition, there is no correlation between experimentally measured or model-predicted  $C_{NO,plat}$  at exhalation flow rates (50 and 250 ml/s) and FVC or  $FEV_1/FVC$  ( $P \geq 0.05$ ).

Figure 6 presents the predicted  $C_{NO,plat}$  (using Eq. 1 with a fixed  $\tau_{res}$  based on a constant exhalation flow

rate) using population mean values from Table 2 (i.e., those determined utilizing a 20-s breath hold) as a function of exhalation flow rate. Experimentally obtained  $C_{NO,plat}$  (mean  $\pm$  SD) at flow rates of 4.2–1,550 from Silkoff et al. (27) are also shown as well as those obtained in the present study at  $\sim$ 50 and  $\sim$ 250 ml/s. The predicted  $C_{NO,plat}$  values are in very close agreement with the measured values from the present study but are lower than those of Silkoff et al. However, this difference is not significant (paired  $t$ -test with  $P > 0.05$ ). The stippled region in Fig. 6 demonstrates the range of flow rates used to estimate the flow-independent parameters. Thus predictions of  $C_{NO,plat}$  outside this region represent extrapolation.

## DISCUSSION

In this study, we further characterized our new technique (34) to determine three flow-independent NO exchange parameters in healthy adults. One or more of these parameters have been estimated in healthy adults by four previous studies (14, 22, 28, 35). Each of these previous studies utilized the governing equations from the same two-compartment model, but each used a different breathing technique to estimate the parameters. All the previous studies utilized breathing techniques that require multiple constant exhalation flows. Table 3 summarizes the results from healthy subjects by these previous studies. The values for the parameters estimated by our new technique are similar to those previously estimated.

It is remarkable to note that, independent of the technique employed, the intrapopulation variance in these parameters (as demonstrated by the 95% confidence interval) within a healthy population is substantial relative to other endogenously produced gases such as  $CO_2$ . The mechanisms underlying this variation are not known. One possibility is simply the size of the subject. For example,  $J_{NO,max}$  and  $D_{NO,air}$  depend on the surface area or volume participating in the exchange process. However, there is no correlation between the estimated values for  $J_{NO,max}$  and  $D_{NO,air}$  with  $V_{air}$  ( $r = 0.25$ ,  $P = 0.48$ , and  $r = 0.30$ ,  $P = 0.40$ , respectively). If one expresses these parameters per unit volume of the airway compartment by dividing by  $V_{air}$ ,  $\Delta\bar{I}_{0.95,i}^m$  actually changes slightly (although not statistically significantly) from 15 and 37% to 16 and 34% for  $J_{NO,max}$  and  $D_{NO,air}$ , respectively.

The correlation of the flow-independent parameters with standard spirometry is of particular interest to the potential clinical application and interpretation of the flow-independent NO parameters. None of the flow-independent parameters is correlated with FVC or  $FEV_1/FVC$ , suggesting that these parameters are characterizing information other than lung volume or airway resistance. Recently, Silkoff et al. (28) reported elevated  $J_{NO,max}$  and  $D_{NO,air}$  in patients with bronchial asthma who had reduced  $FEV_1/FVC$  relative to healthy controls. Of interest is the fact that, within the asthmatic group, Silkoff et al. reported a positive correlation between  $J_{NO,max}$  and  $FEV_1/FVC$ . Thus correlation

Table 2. Flow-independent NO parameters

Subj No.	$J_{NO,max}$ , pl/s	$D_{NO,air}$ , pl·s <sup>-1</sup> ·ppb <sup>-1</sup>	$C_{alv,ss}$ , ppb	$\bar{C}_{tiss,air}$ , ppb	Expiratory Data				$C_{NO,plat}^*$	
					$C_{NO,plat}$ , ppb	$\dot{V}_E$ , ml/s	$C_{NO,plat}$ , ppb	$\dot{V}_E$ , ml/s	50 ml/s	250 ml/s
1	573	5.91	1.91	97	9.67	58.5	4.31	231	12.5	4.13
2	433	2.79	2.69	155	8.89	50.3	4.14	251	11.0	4.38
3	651	5.54	2.51	118	9.25	92.5	2.52	266	14.6	5.03
4	702	2.50	2.71	281	15.5	55.9	5.30	192	16.3	5.48
5	554	7.38	1.05	75.1	9.68	56.4	3.04	270	11.2	3.20
6	853	7.25	1.91	118	13.1	64.4	4.34	249	17.5	5.22
7	696	1.70	2.36	409	14.2	60.0	4.58	253	13.9	5.00
8	825	1.60	2.99	516	19.4	55.3	6.94	208	17.8	6.93
9	515	3.97	3.02	130	9.33	60.2	1.81	231	12.7	5.02
10	598	3.37	3.61	177	14.4	62.7	7.66	253	12.7	5.91
Mean	640	4.20	2.48	208	12.3	61.6	4.46	240	14.0	5.03
SD	133	2.18	0.72	148	3.54	11.6	1.83	25.0	2.47	1.01

Subjects ( $n = 10$ ) performed a 20-s breath hold.  $J_{NO,max}$ , maximum nitric oxide (NO) flux ( $= D_{NO,air} \cdot \bar{C}_{tiss,air}$ );  $D_{NO,air}$ , diffusing capacity of NO in airways;  $C_{alv,ss}$ , steady-state alveolar concentration;  $\bar{C}_{tiss,air}$ , mean tissue concentration in airway;  $C_{NO,plat}$ , plateau NO concentration;  $\dot{V}_E$ , exhalation flow rate. \*Model predicted using the flow-independent parameters for a 20-s breath hold.

between flow-independent NO parameters and airway resistance may depend on the presence of disease.

Although this technique has not characterized the flow-independent NO exchange parameters in populations with lung diseases, the large  $\Delta I_{0.95,i}^m$  suggests that the potential clinical utility in these parameters will likely be intrasubject longitudinal changes. This places critical importance on identifying the intrinsic error of the technique used to estimate the parameters to document a significant change in a parameter from one point in time to another.

We previously demonstrated theoretically that the intramaneuver variability of the parameter estimates would depend on the residence time of the air in the airway compartment (34) and, thus, on the breath-hold

time. Not surprisingly, theory predicted that breath-hold time would affect largely the parameters that characterize the airway compartment ( $J_{NO,max}$  and  $D_{NO,air}$ ), inasmuch as a longer breath-hold time would increase the residence within the airway compartment.

As depicted schematically in Fig. 2, the net flux of NO from the airway compartment is the sum of two terms: 1)  $J_{NO,max}$  and 2)  $-D_{NO,air} \cdot C_{air}$ . Thus, if  $C_{air}$  is small enough (small residence times), the second term is negligible and the flux is entirely characterized by  $J_{NO,max}$  (i.e.,  $D_{NO,air}$  cannot be characterized). Hence, the variability of  $D_{NO,air}$  should be larger than  $J_{NO,max}$ , and the variability in both parameters would be inversely related to breath-hold time. Our data in healthy subjects are consistent with our theoretical prediction.  $\Delta I_{0.95,D_{NO,air}}^m$  is much larger than  $\Delta I_{0.95,J_{NO,max}}^m$ , and both decrease with an increase in breath-hold time from 10 to 20 s. In contrast,  $\Delta I_{0.95,C_{alv,ss}}^m$  should not depend on breath-hold time, inasmuch as the parameter estimate is determined primarily from the data during the decreasing flow rate portion of the breathing maneuver. Our data in healthy subjects demonstrated a slight (although not statistically significant)

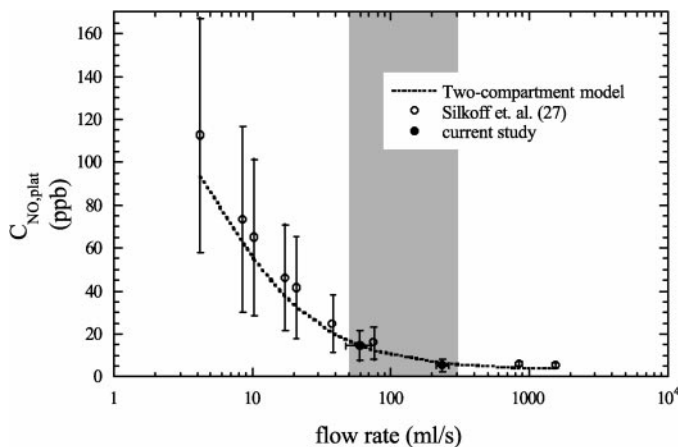


Fig. 6. Plateau concentration ( $C_{NO,plat}$ ) as a function of constant exhalation flow rate.  $\circ$ , Experimentally obtained  $C_{NO,plat}$  values (means  $\pm$  SD) at 4.2–1,550 ml/s flow rate [from Silkoff et al. (27)];  $\bullet$ , values obtained in the present study based on American Thoracic Society and European Respiratory Society guidelines ( $\sim$ 50 and  $\sim$ 250 ml/s). Dashed line, model prediction of  $C_{NO,plat}$  at a constant exhalation flow rate using estimated parameter values determined from the 5 repeated decreasing flow rate maneuvers followed by a 20-s preexpiratory breath hold; stippled region, flow rate range during the decreasing flow maneuver used to estimate the 3 flow-independent parameters in the present study.

Table 3. Comparison of parameter estimates with previous estimates

$J_{NO,max}$ , pl/s	$D_{NO,air}$ , pl·s <sup>-1</sup> ·ppb <sup>-1</sup>	$C_{alv,ss}$ , ppb	$\bar{C}_{tiss,air}$ , ppb	$n$	Ref.
640 (15%)	4.2 (37%)	2.5 (21%)	208 (51%)	10	Present study†
710 (40%)		5.6 (51%)		7	35
1,280 (64%)	5.7 (92%)	2.1 (40%)	224 (20%)*	7	22
1,020 (35%)	6.8 (38%)	5.0 (15%)	150 (48%)	10	28
680 (44%)*	9.2 (22%)	2.0 (50%)	75 (37%)	10	14

Values are means, with 95% confidence interval in parentheses;  $n$ , no. of subjects. \*Calculated using nonlinear error propagation (2). †20-s breath hold.

improvement in  $\Delta \bar{I}_{0.95, C_{alv,ss}}^m$  when the breath-hold time increased from 10 to 20 s.

Breath-hold time did not significantly affect the intrasubject or intrapopulation variability. This finding suggests that the reproducibility of the breathing maneuver does not depend strongly on the breath-hold time, despite the fact that the effort on the part of the subject progressively increases with increasing breath-hold time. Among our 10 healthy subjects, one (*subject 7*) is not able to hold his breath for 45 s. On the basis of these findings, we conclude that breath-hold times >20 s do not provide significant improvement in the accuracy of the parameter estimates.

The accuracy in the estimate of  $J_{NO,max}$  is significantly better than that of  $D_{NO,air}$  and  $C_{alv,ss}$ , as evidenced by smaller intramanuever and intrasubject confidence intervals. The improved ability to estimate  $J_{NO,max}$  is due primarily to the fact that the entire exhalation profile (phases I, II, and III) depends on the value of  $J_{NO,max}$ . In contrast, the estimate of  $D_{NO,air}$  weakly depends on only phases I and II (breath-holding), and the estimate of  $C_{alv,ss}$  depends primarily on phase III (decreasing flow rate portion). An additional source of variance for  $C_{alv,ss}$  is the fact that the limit of resolution of the instrument is  $\sim 1$  ppb, which is similar to the estimated values in healthy subjects (1–4 ppb). The accuracy of the estimate of  $D_{NO,air}$  and  $C_{alv,ss}$  may improve in disease states, in which NO production is increased and the signal-to-noise ratio improves. For example, NO elimination is dramatically increased in bronchial asthma (1, 17, 28), and thus a much larger NO signal should be attained during all phases of the exhalation profile, but particularly during phases I and II, which reflect production of NO from the airways. In addition, it has recently been demonstrated that alveolar concentration levels may increase two- to threefold in alveolitis (20), which would also increase the signal-to-noise ratio and thus reduce  $\Delta \bar{I}_{0.95, C_{alv,ss}}^m$ .

The intramanuever confidence interval is an a priori estimate of the uncertainty in the parameter estimate made from a single breathing maneuver. Thus  $\Delta \bar{I}_{0.95,i}^m$  has potential utility as a screening tool to accept or reject a given profile. For example, for a given individual maneuver, if  $\Delta \bar{I}_{0.95,i}^m$  is >100%, one cannot conclude with 95% confidence that the estimated parameter is statistically different from zero (confidence interval includes zero). Thus one might choose to eliminate this maneuver and repeat the maneuver until  $\Delta \bar{I}_{0.95,i}^m$  is <100%. The population mean values for  $\Delta \bar{I}_{0.95,i}^m$  are 26, 168, and 81% for  $J_{NO,max}$ ,  $D_{NO,air}$ , and  $C_{alv,ss}$ , respectively, for a 20-s breath hold. This highlights that  $J_{NO,max}$  can be estimated with the highest certainty and  $D_{NO,air}$  with the least certainty.  $D_{NO,air}$  remains potentially useful, inasmuch as the variation within and between subjects can be significant; however, multiple single maneuvers may be required to estimate its value within a desired accuracy.

There are several possible confounding variables in the technique that may impact the parameter estimates. During inspiration, NO from the nasal cavity may be absorbed through the nasopharynx and the soft

palate, which was not closed. Although this additional NO would be absorbed in the alveolar region during the breath hold and thus would not likely impact  $C_{alv,ss}$ , it may artificially increase the NO concentration in the airway compartment during the breath hold. If this amount of NO were significant, we would anticipate a larger effect at the shorter breath-hold times, where the NO entrained from the nasal cavity would be a larger fraction of the total at the end of the breath hold; thus we would observe an inverse dependence between  $D_{NO,air}$  and/or  $J_{NO,max}$  and the breath-hold time. This concept can be demonstrated quantitatively by demonstrating that the relative sensitivity (34) of  $D_{NO,air}$  and  $J_{NO,max}$  to the initial concentration is an inverse function of the breath-hold time. Experimentally, these two parameters do not depend on the breath-hold time (Fig. 4); thus it is unlikely that nasal NO is a significant confounding variable.

A second possible source of error is performing the spirometric breathing maneuvers before the NO breathing maneuver. Silkoff et al. (29) and Deykin et al. (8, 9) recently demonstrated that spirometry can depress exhaled NO levels by 10–36% from the baseline in healthy subjects and subjects with asthma. This may impact one or more of the flow-independent parameters and should be considered in any future studies.

A third possible source of error is the estimate in the airway compartment volume with the use of the subjects' ideal body weight (pounds) plus age (years). The estimate of  $D_{NO,air}$  is a positive function of the estimate of  $V_{air}$ , whereas  $J_{NO,max}$  and  $C_{alv,ss}$  are nearly independent of  $V_{air}$  (34). The present technique could be combined with a nitrogen washout (Fowler method) to estimate dead space (23, 25); however, the accuracy of the Fowler method is compromised by the presence of diseases that impact emptying patterns. The dependence of  $D_{NO,air}$  on  $V_{air}$  may explain some of the inter-subject variability and suggests that intrasubject longitudinal changes in the flow-independent parameters may have the greatest clinical utility.

Finally, we previously demonstrated that, during a vital capacity maneuver at a constant exhalation flow rate that includes a 15-s breath hold (35), the slope of phase III for the NO exhalation profile has a statistically negative slope. This could be due to a decreasing alveolar concentration and/or a decreasing flux of NO from the airway compartment. It is not likely due to a decreasing alveolar concentration, inasmuch as our laboratory previously demonstrated that the alveolar diffusing capacity for NO decreases with decreasing lung volume, which would serve to increase the alveolar concentration (33).

In contrast, the airways are somewhat flexible and will distend with inspiration and contract with expiration. During expiration, the airways contract slightly, which may decrease  $V_{air}$  as well as the surface area for exchange of NO between the airway wall and gas phase. A decrease in the surface area would decrease  $D_{NO,air}$ . Interestingly, a decrease in  $V_{air}$  during expiration has no impact on the model equations, inasmuch



as the concentrating effect of the smaller volume is precisely offset by the reduced residence time in the smaller volume (mathematical proof not shown). In addition, the loss of NO to the passing gas stream during expiration may decrease  $\dot{C}_{\text{tiss,air}}$ , which, in turn, would decrease the flux of NO from the airway wall (and  $J_{\text{NO,max}}$ ) and create a negative phase III slope. Thus the flow-independent parameters may not be constant during a vital capacity maneuver but may depend on factors such as lung volume. Further investigation is necessary before it is known whether nuances such as lung volume need to be considered. Hence, the simplifying assumptions of the two-compartment model require that the flow-independent parameters be interpreted as global descriptors of NO exchange dynamics.

In summary, we have quantified flow-independent parameters ( $J_{\text{NO,max}}$ ,  $D_{\text{NO,air}}$ ,  $\dot{C}_{\text{tiss,air}}$ , and  $C_{\text{alv,ss}}$ ) in a healthy adult population utilizing a technique that employs a breath hold followed by a decreasing flow rate maneuver. Mean population values compare favorably with previous reports, which utilized techniques requiring multiple breathing maneuvers. There is no correlation between the flow-independent NO parameters and FVC or FEV<sub>1</sub>/FVC, suggesting that these NO parameters are providing different information regarding lung function. Importantly, we have also quantified the intramanuever, intrasubject, and intrapopulation confidence intervals in healthy adults for each of the parameters. We conclude that there is significant variation within the population of healthy adults in terms of the magnitude of the parameters as well as the confidence interval. Thus longitudinal tracking within a given subject may provide the most useful information. In addition,  $J_{\text{NO,max}}$  can be estimated with the highest level of certainty and, therefore, may be the most useful parameter to monitor in disease states. Future studies must quantify these parameters in key inflammatory diseases such as bronchial asthma, cystic fibrosis, and chronic obstructive pulmonary disease before their potential clinical utility is known.

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