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Authors
SHIN, HYE-WON
ROSE-GOTTRON, CHRISTINE M
COOPER, DAN M
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Impact of High-Intensity Exercise on Nitric Oxide Exchange in Healthy Adults

HYE-WON SHIN[2], CHRISTINE M. ROSE-GOTTRON[4], DAN M. COOPER[3,4], MARYANN HILL[3,4], and STEVEN C. GEORGE[1,2]

[1]Department of Biomedical Engineering, [2]Department of Chemical Engineering and Materials Science, [3]Department of Pediatrics, and [4]The General Clinical Research Center, University of California, Irvine, Irvine, CA

ABSTRACT

SHIN, H.-W., C. M. ROSE-GOTTRON, D. M. COOPER, M. HILL, and S. C. GEORGE. Impact of High-Intensity Exercise on Nitric Oxide Exchange in Healthy Adults. *Med. Sci. Sports Exerc.*, Vol. 35, No. 6, pp. 995–1003, 2003. **Purpose:** After exercise, exhaled NO concentration has been reported to decrease, remain unchanged, or increase. A more mechanistic understanding of NO exchange dynamics after exercise is needed to understand the relationship between exercise and NO exchange. **Methods:** We measured several flow-independent NO exchange parameters characteristic of airway and alveolar regions using a single breath maneuver and a two-compartment model (maximum flux of NO from the airways, J'_{awNO}, $pL \cdot s^{-1}$; diffusing capacity of NO in the airways, D_{awNO}, $pL \cdot s^{-1} \cdot ppb^{-1}$; steady state alveolar concentration, $C_{alv,ss}$, ppb; mean airway tissue NO concentration, C_{awNO}, ppb), as well as serum IL-6 at baseline, 3, 30, and 120 min after a high-intensity exercise challenge in 10 healthy adults (21–37 yr old). **Results:** D_{awNO} (mean ± SD) increased (37.1 ± 44.4%), whereas J'_{awNO} and C_{awNO} decreased (−7.27 ± 11.1%, −26.1 ± 24.6%, respectively) 3 min postexercise. IL-6 increased steadily after exercise to 481% ± 562% above baseline 120 min postexercise. **Conclusion:** High-intensity exercise acutely enhances the ability of NO to diffuse between the airway tissue and the gas phase, and exhaled NO might be used to probe both the metabolic and physical properties of the airways. **Key Words:** NO, CYTOKINES, PARAMETER ESTIMATION, GAS EXCHANGE

Nitric oxide (NO) performs many important functions in the lungs and can be detected in the exhaled breath of humans. Inflammatory diseases such as asthma and cystic fibrosis alter exhaled NO levels, which has generated interest in utilizing exhaled NO as a noninvasive marker of lung inflammation (2). However, the exchange dynamics of NO are markedly different from the respiratory gases whose exchange occurs predominantly in the alveolar region. In contrast, NO exchange occurs in both alveolar and airway compartments, and is thus highly dependent on the exhalation flow rate (26,34). This feature of NO exchange has confounded interpretation of exhaled NO in a variety of clinical and physiological settings. We have addressed this feature of NO exchange is several prior studies (23,24,32,33) by characterizing both airway and alveolar compartment contributions to exhaled NO with a series of flow-independent NO exchange parameters.

Given the nature of NO exchange dynamics, and the multi-system physiological responses to exercise, it is not surprising that there are inconsistencies in the reports of the impact of exercise on exhaled NO. After exercise, exhaled NO concentration has been reported to be increased (3), unchanged (12), or decreased (6,15,16,19,29). Recently, Scollo et al. (21) reported no significant change in exhaled NO concentration up to 18 min after an exercise challenge in children. However, De Gouw et al. (7) extended exhaled NO monitoring for 30 min in healthy adults and observed a small decrease in exhaled NO concentration shortly after the exercise (<5 min) and an increase >20 min after exercise. One confounding variable in these previous studies is the mild inflammatory response induced by exercise (5,17,18). It has previously been demonstrated that animal models of acute systemic inflammation can induce an increase in exhaled NO (9,27).

By distinguishing alveolar and airway contributions to exhaled NO, our approach provides greater specificity than exhaled concentration alone and thus may be able to address several unresolved questions regarding the impact of exercise on NO exchange. The primary aim of the current study was to determine a more mechanistic understanding of NO exchange after acute high-intensity exercise. We characterized NO exchange using a series of flow-independent NO exchange parameters (32,33) for 2 h after high-intensity exercise in healthy adults. In addition, we estimated the degree of systemic inflammation after exercise by measuring the serum level of the proinflammatory cytokine IL-6.

TABLE 1. Physical characteristics of subjects.

Subject	Gender	Age (yr)	Height (inches)	Weight (lb)	Ideal Body Weight (lb)	V_{air} (mL)
1	M	36	69	145	145	181
2	M	24	72	175	167	191
3	M	21	68	153	152	173
4	F	24	70	150	147	171
5	F	22	61	125	117	139
6	M	23	66	157	145	168
7	F	34	59	103	111	145
8	F	23	61	117	119	142
9	M	37	70	165	160	197
10	F	26	65	139	130	156
Mean		27	66	143	139	166

METHODS

Subjects. Ten nonsmoking healthy adults (ages 21–37 yr) were recruited to participate in this study. Subjects were categorized as healthy and free of lung diseases on the basis of a brief medical history and standard spirometry ($FEV_1/FVC > 80\%$). Subject characteristics are listed in Table 1. The airway compartment volume (V_{aw}, mL) was estimated from the subjects' ideal body weight plus their age (yr) as previously described (33) and is needed for the flow-independent NO parameter estimation as described below. Subjects were asked not to exercise for at least 72 h and refrain from any food for at least 3 h before the study. The protocol was approved by the Institutional Review Board at the University of California, Irvine, and informed written consent was obtained from all the subjects before the experiments.

Exercise challenge. The exercise test was performed using a treadmill. Each subject performed a high-intensity exercise challenge in which the target intensity was 90% of the predicted maximum heart rate (220 − age in years) for 20 min. Heart rate was continuously monitored with a three-lead electrocardiogram. Exhaled NO profiles followed by pulmonary function testing were performed as baseline, 3, 30, and 120 min postexercise challenge.

Cytokine assay. Serum level of IL-6 was measured at baseline, and exercise (3, 30, and 120 min) using ELISA with a commercially available assay kit from R&D systems (Quantikine High Sensitivity Kit, R&D system; Minneapolis, MN).

Spirometry. Forced vital capacity (FVC) and FEV_1/FVC were measured in triplicate in all subjects (V_{max}229; SensorMedics, Yorba Linda, CA) at baseline, 3, 30, and 120 min postexercise challenge after measuring exhaled NO.

Exhaled NO measurement. NO exchange was characterized in two ways. First, three repetitions, separated by approximately 15–30 s, of a 20-s preexpiratory breathhold followed by a decreasing exhalation flow rate (from ~6% to ~1% vital capacity per second) maneuver were performed to determine flow-independent NO exchange parameters. Second, baseline plateau NO concentration (C_{NOplat}) at a targeted constant exhalation flow rate ($\dot{V}_E$) of ~50 mL·s^{-1} and ~250 mL·s^{-1} was collected according to the guidelines of the American Thoracic Society (ATS)

and European Respiratory Society (ERS), respectively. A noseclip was worn during all breathing maneuvers, subjects inspired NO-free air (Fig. 1), and both techniques were implemented at baseline and postexercise challenge (3, 30, and 120 min).

During the breathhold maneuver, a positive pressure of > 5 cm H_2O was maintained to prevent nasal contamination, and the NO sampling line sampled air from an NO-free reservoir. Figure 1 presents a schematic of the experimental apparatus, a detailed description of which has been described previously (33). 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. Flow rate was facilitated via a Starling resistor (Hans Rudolph, Kansas City, MO) with a variable resistance.

A previously described two-compartment model and nonlinear least squares minimization method were used to estimate the key flow-independent NO parameters.
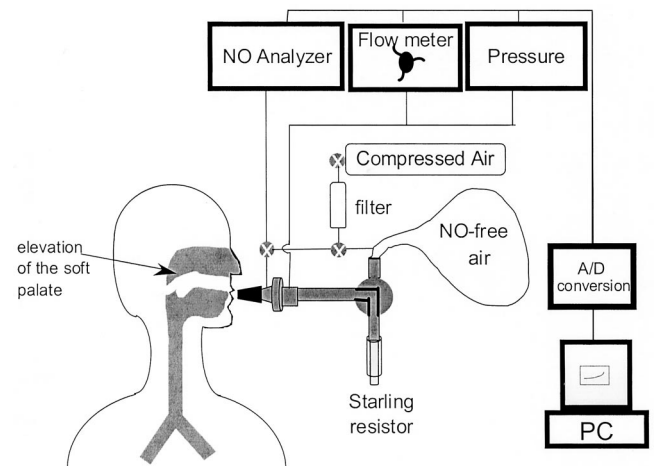

FIGURE 1—Schematic of the experimental setup used to collect the exhalation profiles. The flow, pressure, and NO analog signals are captured by the analytical instruments and converted to a digital signal. A series of valves allows NO-free air to be stored in a Mylar bag for inspiration. During the breathhold, the NO analyzer samples from the NO-free air reservoir, and the subject maintains a positive pressure of > 5 cmH₂O by attempting to exhale against a closed valve. As exhalation begins, the NO analyzer then samples from the exhalate, and the flow rate is manipulated by a variable Starling resistor while the expiratory effort of the subject remains constant.
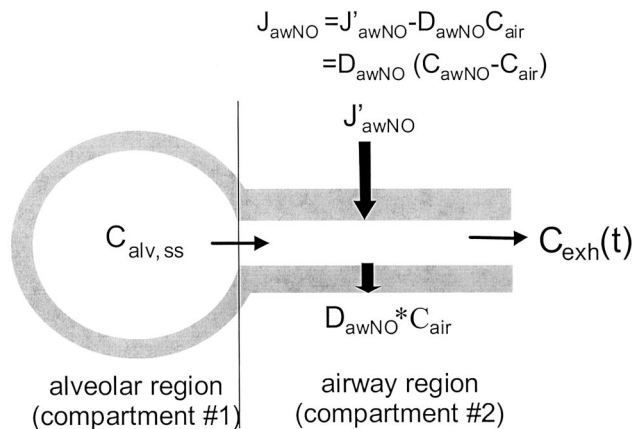
$$J_{awNO} = J'_{awNO} - D_{awNO}C_{air}$$
$$= D_{awNO}(C_{awNO} - C_{air})$$

FIGURE 2—Schematic of two-compartment model used to describe NO exchange dynamics. Exhaled NO concentration, C_{exh}, depends on three flow-independent parameters: maximum flux of NO from the airways (J'_{awNO}, $pL \cdot s^{-1}$), diffusing capacity of NO in the airways (D_{awNO}, $pL \cdot s^{-1} \cdot ppb^{-1}$), and steady-state alveolar concentration ($C_{alv,ss}$, ppb).

Figure 2 is a simple schematic of the two-compartment model and flow-independent parameters. Detailed description of the parameters has been previously presented (33), and only the salient features will be presented herein.

Briefly, J_{awNO} ($pL \cdot s^{-1}$) is defined as the flux of NO from the airways, which represents NO transferred from the airway tissue to the gas phase on exhalation. J_{awNO} is a linear function of the gas phase concentration (C_{air}) and is characterized by J'_{awNO} and D_{awNO} ($J_{awNO} = J'_{awNO} - D_{awNO}C_{air}$). Alternatively, J_{awNO} can be expressed as proportional to the concentration difference between the airway tissue (C_{awNO}) and the gas stream (C_{air}) with the proportionality coefficient of D_{awNO} ($J_{awNO} = D_{awNO}(C_{awNO} - C_{air})$).

J'_{awNO} is the maximum flux of NO from the airway tissue which is equal to the airway compartment flux if the gas phase concentration, C_{air}, were zero. C_{awNO} represents the mean (over radial position) airway tissue concentration and is simply the ratio J'_{awNO}/D_{awNO}. D_{awNO} can be interpreted as a conductance for mass transfer of NO between the airway tissue and the gas phase. The alveolar region is characterized by the steady state alveolar concentration $C_{alv,ss}$.

To control for small inter- and intra-subject variability in $\dot{V}_E$ during the constant exhalation flow rate maneuver, we also used the two-compartment model to predict C_{NOplat} at exactly 50 and 250 $mL \cdot s^{-1}$. We have previously demonstrated (23,24) that the two-compartment model can accurately predict C_{NOplat} using the flow-independent parameters using the following relationship that has been previously derived (32,33) from a mass balance for NO in the alveolar and airway compartments:

$$C_{NOplat} = \left(C_{alv,ss} - \frac{J'_{awNO}}{D_{awNO}}\right)e^{-\frac{D_{awNO}}{\dot{V}_E}} + \frac{J'_{awNO}}{D_{awNO}} \quad (1)$$

Thus, once the flow-independent parameters were estimated from the breathhold maneuver, they can be inserted into Eq. 1 and be used to predict C_{NOplat} at precisely $\dot{V}_E$ of 50 and 250 $mL \cdot s^{-1}$.

A rapid-response chemiluminescence NO analyzer (NOA280, Sievers, Inc., Boulder, CO) with 7.4 mm Hg operating reaction cell pressure and sampling flow rate of 200 $mL \cdot min^{-1}$ was used to measure the exhaled NO concentration. Calibration of the NO analyzer was performed using a certified NO gas (45 ppm in N_2, Sievers, Inc., Boulder, CO) and NO-free air was obtained by passing compressed air through a NO filter (Sievers, Inc., Boulder, CO) before the collection of the NO exhalation profile. The flow rate and pressure signals were measured using a pneumotachometer (RSS100, Hans Rudolph Inc., Kansas City, MO), which was calibrated daily and was set to provide the flow in units of STPD and pressure in units of mm Hg. The analog signals of NO, flow and pressure were digitized using an A/D card at a rate of 50 Hz and stored on a PC for further analysis.

Statistical analysis. Repeated measures ANOVA was used to test differences among means of the NO exchange parameters and also the cytokines at baseline and postexercise challenge at 3, 30, and 120 min. Contrasts similar to paired t-tests were used to characterize when differences from baseline were largest (baseline to the 3 min value, baseline to 30 min postexercise, etc.). For both the NO exchange parameters and the cytokines, differences were computed from baseline to the other time points and then correlations were computed among these differences.

RESULTS

All subjects were able to complete the 20 min of high-intensity exercise without complication. In addition, all subjects were able to achieve the target intensity within 3 min of beginning the exercise. FVC and FEV_1/FVC at baseline, 3, 30, and 120 min postexercise challenge are presented in Table 2A. No significant postexercise changes in FVC and FEV_1/FVC were observed ($P > 0.05$).

The composite exhalation profile (single breath maneuver with 20-s preexpiratory breathhold) for NO for the 10 subjects at baseline and 3 min postexercise is shown in Figure 3. Taking the mean concentration at equal exhaled volumes for the 10 subjects generated these profiles. It is evident that less NO is exhaled 3 min postexercise. This can be seen by the reduced peak height (37 ppb compared with 23 ppb) in phase I and II, and also the flatter slope in phase III of the composite postexercise profile relative to baseline. The boundary between phase II and III is defined by the inflection point (i.e., zero slope) of the exhalation profile as previously described (33). These experimentally observed changes in exhaled concentration are reflected in changes in the flow-independent NO exchange parameters.

Flow-independent NO parameters (J'_{awNO}, D_{awNO}, $C_{alv,ss}$, and C_{awNO}) are presented in Figures 4–7 with the percent change in each parameter in the upper panel (A) and the response of each individual relative to baseline at 3, 30, and 120 min postexercise in the lower panel (B). Significant differences among means over time were identified from the

TABLE 2. Pulmonary functions for baseline, 3, 30, and 120 min postexercise challenge.

Subject	FVC (L, % predicted)								FEV₁/FVC			
	Base	3-PostEX	30-PostEX	120-PostEX	Base	3-PostEX	30-PostEX	120-PostEX	Base	3-PostEX	30-PostEX	120-PostEX
1	4.76	4.8	4.71	4.52	97	98	96	92	80	82	83	82
2	5.76	5.67	5.89	5.8	101	100	104	102	84	90	87	87
3	4.69	4.55	4.66	4.65	96	93	96	96	93	93	92	93
4	4.76	4.61	4.63	4.61	111	107	108	107	81	81	80	80
5	3.31	3.28	3.29	3.37	100	99	99	102	83	84	80	73
6	4.21	4.11	4.3	4.14	89	87	91	88	88	91	86	89
7	3.61	3.35	3.26	3.37	125	116	113	117	86	89	89	87
8	2.53	2.58	2.62	2.63	76	77	78	79	85	84	82	81
9	4.66	4.63	4.67	4.72	91	90	91	92	86	84	85	86
10	4.67	4.68	4.58	4.62	127	127	124	125	80	79	79	80
Mean	4.30	4.23	4.26	4.24	101	99.4	100	100	85	86	84	84
SD	0.92	0.91	0.95	0.90	15.9	14.5	12.9	13.7	4.01	4.72	4.27	5.71

PostEX, postexercise.

repeated measure ANOVA for J'_{awNO} ($F = 4.64$, $P = 0.01$) and C_{awNO} ($F = 5.91$, $P = 0.003$). J'_{awNO}, D_{awNO}, and C_{awNO} were significantly different compared with the baseline ($P < 0.05$) 3 min postexercise. D_{awNO} was elevated ($37.1 \pm 44.4\%$), whereas J'_{awNO} and C_{awNO} were decreased ($-7.27 \pm 11.1\%$, $-26.1 \pm 24.6\%$, respectively). At 30 and 120 min postexercise, all flow-independent parameters were not different from baseline. None of the flow-independent parameters had any significant correlation with standard indices of lung volume (FVC) or airway obstruction (FEV₁/FVC).

Experimentally measured and model-predicted (Eq. 1) plateau NO (C_{NOplat}) concentration at targeted $\dot{V}_E$ of 50 and 250 mL·s⁻¹ are presented in Table 3. Mean experimentally measured baseline C_{NOplat} ($\pm$ SD) were 9.78 ± 6.43 ppb and 3.03 ± 2.17 ppb at a mean $\dot{V}_E$ equal to 52.9 mL·s⁻¹ (target 50 mL·s⁻¹) and 244 mL·s⁻¹ (target 250 mL·s⁻¹), respectively. Mean model-predicted (Eq. 1) baseline C_{NOplat} ($\pm$ SD) were 9.91 ± 6.02 ppb and 3.46 ± 2.43 ppb at $\dot{V}_E$

equal to exactly 50 mL·s⁻¹ and 250 mL·s⁻¹, respectively. There was no significant change in C_{NOplat} postexercise at either $\dot{V}_E$ using the experimentally measured values or the model-predicted values ($P > 0.05$).

Systemic circulating IL-6 levels increased significantly and steadily after exercise in all subjects ($92.3 \pm 79.6\%$, $256 \pm 211\%$, and $481 \pm 562\%$ above baseline at 3, 30, and 120 min postexercise, respectively). All differences for IL-6 were significantly different from baseline but did not correlate with changes in the flow-independent NO parameters.

DISCUSSION

This is the first study to examine flow-independent NO exchange parameters after a high-intensity exercise challenge in healthy subjects. We found differences in three flow-independent NO parameters (J'_{awNO}, D_{awNO}, and C_{awNO}) 3 min postexercise, despite the fact that exhaled concentration (C_{NOplat}) was unchanged. These results lead us to conclude that exercise acutely enhances the ability of NO to diffuse between the airway tissue and the gas phase. This is particularly interesting as it suggests that exhaled NO, an endogenously produced molecule associated with inflammatory changes in the lungs, may also be used to probe physical properties of the airways.

We have previously derived approximate analytical expressions for the steady state values of J'_{awNO}, D_{awNO} and C_{awNO} based on mass balances in the airway tissue volume (31,32):

$$J'_{awNO} = A_{aw}S_{tiss,aw}\sqrt{\mathscr{D}_{NO,tiss}/k}[\coth(\xi) - (1 - \coth(\xi))\exp(-\xi)] \quad (2)$$

$$D_{awNO} = \frac{A_{aw}\lambda_{tiss,air}\sqrt{\mathscr{D}_{NO,tiss}k}}{\tanh(\xi)} \quad (3)$$

$$C_{awNO} = \frac{S_{tiss,aw}}{k\lambda_{tiss,air}}[1 - (\tanh(\xi) - 1)\exp(-\xi)] \quad (4)$$

where $S_{tiss,aw}$ is the production rate of NO per unit volume of airway tissue (mL NO·mL⁻¹ tissue), $\lambda_{tiss,air}$ is the tissue:air partition coefficient of NO (solubility of NO in tissue), k (s⁻¹) is the first-order rate constant that characterizes the rate of chemical consumption by substrates such as superoxide, A_{aw} (cm²) is the surface area avail-
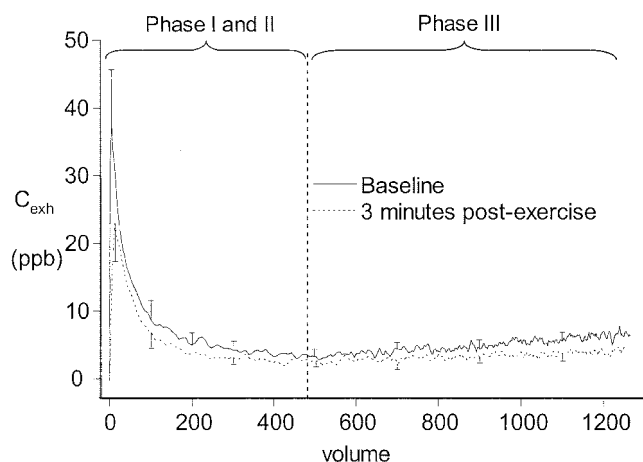

FIGURE 3—Composite exhalation profiles from the single breath technique (i.e., inhalation to TLC followed by a 20-s breathhold and a decreasing exhalation flow rate) in the 10 subjects at baseline and at 3 min postexercise. *Error bars* represent SEM at the peak concentration and then at 200-mL intervals. There is less NO exhaled postexercise as seen by the smaller peak in phase I and II of the exhalation profile, and the positive slope in phase III is also reduced. These changes in exhaled concentration are reflected in changes in the flow-independent NO exchange parameters.
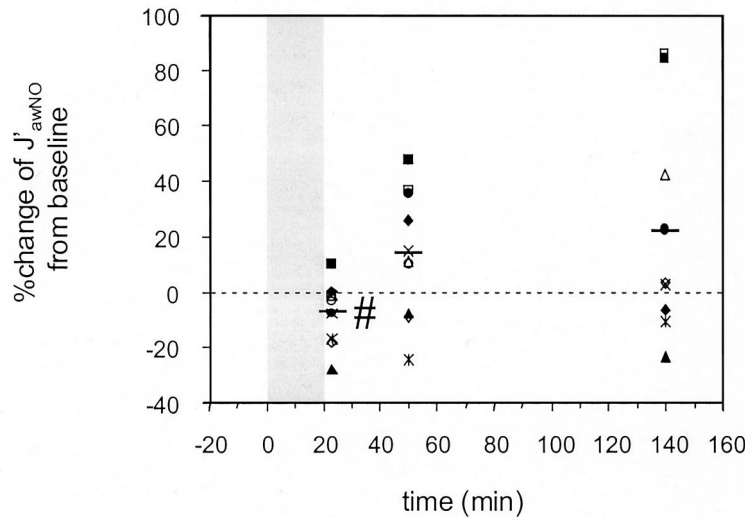
FIGURE 4—A. Percent change of J'_{awNO} from baseline to 3, 30, and 120 min postexercise challenge in 10 healthy subjects. *Open* and *closed symbols* represent percent change of J'_{awNO} in each individual, and *bar* indicates mean percent change. **B.** Individual levels of J'_{awNO} with corresponding population mean (*horizontal bar*) from baseline to 3 min postexercise (base-3postEX), from baseline to 30 min postexercise (base-30postEX), and from baseline to 120 min postexercise (base-120postEX).
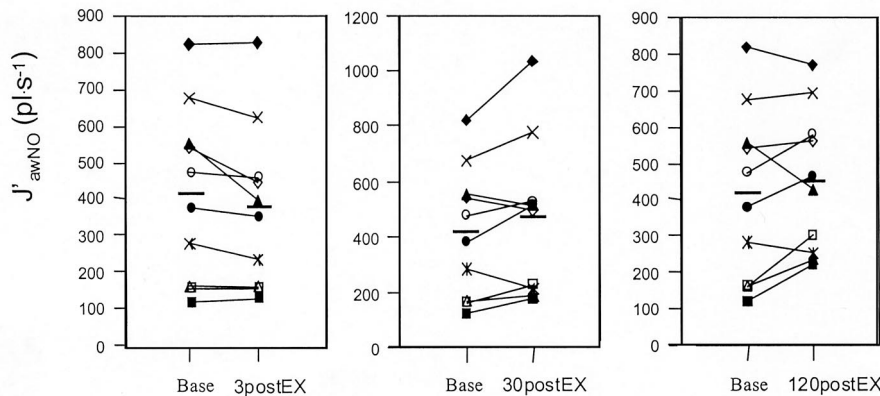
able for diffusion between the airway wall and the gas phase in the airway lumen, $\mathscr{D}_{NO,tiss}$ ($cm^2 \cdot s^{-1}$) is the molecular diffusivity of NO in the tissue (an index of the ease at which NO can be transported by diffusion in the tissue), $\xi = L_{tiss}/\sqrt{\mathscr{D}_{NO,tiss}/k}$, and L_{tiss} is the thickness of the tissue layer. Although approximations, Eqs. 2–4 can provide insight into both the specific variables and their relative impact on the flow-independent NO parameters.

The reduced peak height and elimination of NO in phase I and II of the exhalation profile corresponds to the increase in D_{awNO} 3 min postexercise. An increase in D_{awNO} reduces the net flux of NO into the airway space during the breathhold (recall, $(J_{awNO} = J'_{awNO} - D_{awNO} \cdot C_{air})$. During the breathhold, the product $D_{awNO}C_{air}$ becomes significant relative to J'_{awNO} such that D_{awNO} can be estimated (33). For example, at baseline, the mean value of $D_{awNO}C_{air}$ is approximately $2.2\ pL \cdot s^{-1} \cdot ppb^{-1} \cdot 37$ ppb $= 81.4\ pL \cdot s^{-1}$, which is approximately 20% of the mean value of J'_{awNO} ($\sim 400\ pL \cdot s^{-1}$). From Eq. 3, D_{awNO} is a positive function of A_{aw}, $\lambda_{tiss:air}$, $\mathscr{D}_{NO,tiss}$, and k, and is an inverse function of L_{tiss}. Thus, the increase in D_{awNO} 3 min postexercise may be due to alterations in any of these parameters.

An inflammatory response can enhance bronchial microvascular leakage (38). The plasma exudate has a higher water content than interstitial tissue, which could decrease the solubility of NO (a lipophilic molecule) in the airway wall (i.e., decrease $\lambda_{tiss:air}$) and thus decrease D_{awNO}. In addition, the plasma exudate may increase the thickness of the airway wall (increase in L_{tiss}) and decrease D_{awNO}. Conversely, molecular diffusion of small solutes is reduced in tissue relative to water; thus, a higher water content in the airway wall would increase $\mathscr{D}_{NO,tiss}$ and thus increase D_{awNO}. Indeed, the level of exercise in our study induced a systemic inflammatory response as indicated by the steady increase in serum IL-6, which is consistent with previous reports (5,17,18). However, our data do not allow us to determine the specific temporal relationship between possible inflammatory changes in the airways and changes in flow-independent NO exchange parameters.

An alteration in k is possible due to the increased production of oxygen radicals (e.g., superoxide) during exercise. This follows from increased antioxidant activity (such as superoxide dismutase, a known superoxide scavenger) after exercise in skeletal muscle (1,13), mitochon-
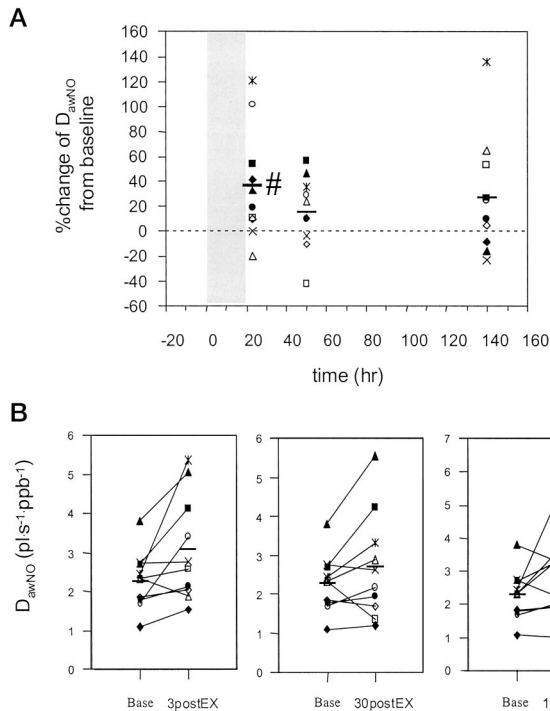
FIGURE 5—A. Percent change of D_{awNO} from baseline to 3, 30, and 120 min postexercise challenge in 10 healthy subjects. *Open* and *closed symbols* represent percent change of D_{awNO} in each individual, and bar indicates mean percent change. **B.** Individual levels of D_{awNO} with corresponding population mean (*horizontal bar*) from baseline to 3 min postexercise (base-3postEX), from baseline to 30 min postexercise (base-30postEX), and from baseline to 120 min postexercise (base-120postEX).

FIGURE 6—A. Percent change of $C_{alv,ss}$ from baseline to 3, 30, and 120 min postexercise challenge in 10 healthy subjects. *Open* and *closed symbols* represent percent change of $C_{alv,ss}$ in each individual, and *bar* indicates mean percent change. **B.** Individual levels of $C_{alv,ss}$ with corresponding population mean (*horizontal bar*) from baseline to 3 min postexercise (base-3postEX), from baseline to 30 min postexercise (base-30postEX), and from baseline to 120 min postexercise (base-120postEX).

dria (10), plasma (14), and lung tissue (37). The enhanced D_{awNO} is due to an increase in the radial concentration gradient of nitric oxide and is a well-known phenomenon in chemical reaction engineering and transport phenomena (4).

Exercise has also been reported to cause mild postexercise bronchodilation in healthy adults, which can be manifested in an increase in FEV_1/FVC (7,28). Bronchodilation could conceivably increase the surface area for diffusion and thus increase D_{awNO}. We did not observe any significant changes in FEV_1/FVC in our healthy subjects postexercise, which suggests there was not any significant bronchodilation. Thus, the observed increase in D_{awNO} is likely due to a combination of factors that may alter several chemical and physical properties of the airway wall.

The elimination rate (product of flow rate and exhaled concentration) of NO from the lungs during exercise has been reported to be increased during exercise by several groups of investigators (3,6,12,15,19,20). The exhaled concentration during exercise remains the same or decreases slightly. Thus, the increased rate of elimination is thought to be due primarily to an increased ventilation rate which either removes NO from tissue stores or decreases the relative fraction of endogenous NO lost to the blood in the pulmonary circulation. Our finding of a decreased C_{awNO} 3 min postexercise is consistent with enhanced loss of NO from airway tissue stores during exercise. This may be due to the increased ventilation rate
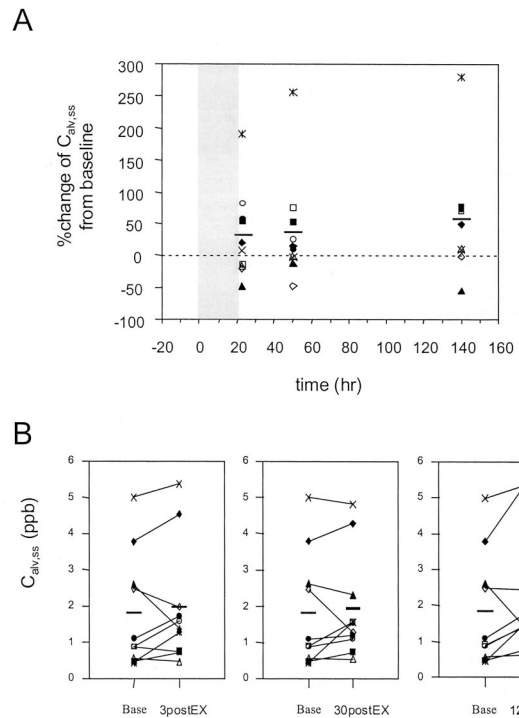
or due to the increase in D_{awNO}. Recall that C_{awNO} is the ratio of J'_{awNO}/D_{awNO}. The fact that C_{awNO} returns to baseline at 30 min suggests that airway tissue stores have returned to baseline levels in this time frame; however, this cannot discriminate between the effects of ventilation rate and D_{awNO} as the latter also returns to baseline in this time frame.

J'_{awNO} is the product of $D_{awNO}C_{awNO}$, and the observed change 3 min postexercise is small in magnitude. This may be due to the fact that the changes in D_{awNO} and C_{awNO} at 3 min postexercise tend to cancel each other— small airway tissue concentration and thus smaller driving force for diffusion, but a reduced resistance to diffusion. Nevertheless, during relatively high exhalation flow rates (i.e., those observed in phase III or > 50 mL·s^{-1}), J_{awNO} can be approximated by J'_{awNO} (32,33) as the product $D_{awNO}C_{air}$ is relatively small compared with J'_{awNO} (e.g., $D_{awNO}C_{air}$ is approximately 2.2 pL·s^{-1}·ppb^{-1}·5 ppb = 11 pL·s^{-1} compared with J'_{awNO} of ~ 400 pL·s^{-1} at baseline). The slope of phase III during the decreasing flow rate maneuver becomes flatter as the airway compartment contribution to the exhaled concentration decreases. The lower flow rates toward the end of exhalation increase the residence time of the air in the airway compartment, resulting in a higher exhaled concentration. Thus, the reduced slope of phase III in the composite profile (Fig. 3) corresponds to a reduced J'_{awNO}.
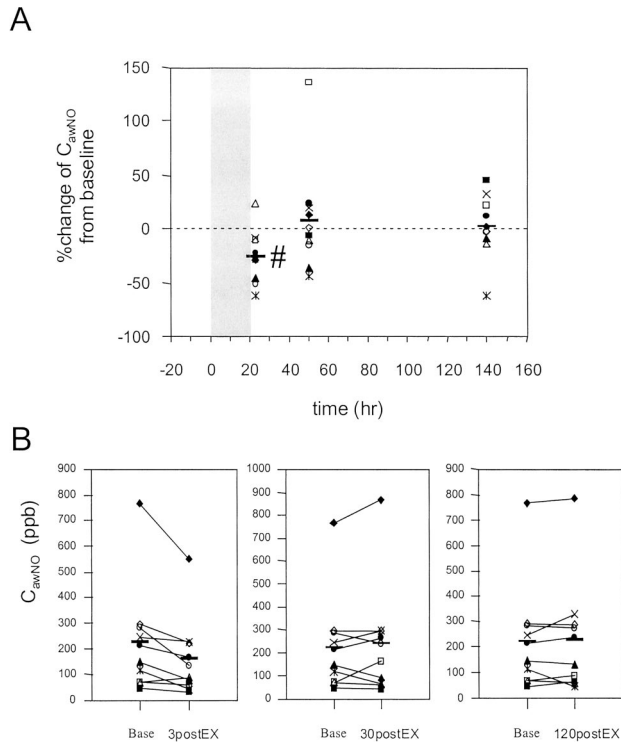
changes. In our study, there was a decrease and an increase at $\dot{V}_E = 50$ mL·s^{-1} in the mean value of C_{NOplat} at 3 (-8%) and 30 min (11%) postexercise, albeit small in magnitude and not statistically significant. However, a closer look at the 10 subjects reveals that between 7 and 9 demonstrated an increase in C_{NOplat} at 30 min depending on the flow rate and whether the model-predicted value of C_{NOplat} is used. For example, at $\dot{V}_E = 50$ mL·s^{-1}, if subject 6 is removed, the remaining nine subjects all had an increase in C_{NOplat} at 30 min which is highly significant ($P < 0.01$). There is no reason to exclude subject 6 in our study as all other indices of lung function were normal. This result highlights the important intersubject variability in NO exchange dynamics, which has been previously reported (23,24), and the relatively small changes in exhaled NO concentration observed after exercise.

At $\dot{V}_E = 250$ mL·s^{-1}, our study revealed an increase in C_{NOplat} at both 3 and 30 min (18% and 19%, respectively), although these changes were not significant. The increase in C_{NOplat} at 3 min contrasts with the trend observed at the lower flow rates in our study and the decrease observed by De Gouw et al. (7). At higher flow rates, the residence time of each gas bolus in the airway compartment is less; thus, the exhaled concentration (i.e., C_{NOplat}) is more dependent on the alveolar concentration. Although the observed changes in $C_{alv,ss}$ were not significant, the trend at 30 min was for $C_{alv,ss}$ to increase and thus might account for the observed increase in C_{NOplat} at $\dot{V}_E = 250$ mL·s^{-1}, which is also higher than that used by De Gouw et al. (7).

The two-compartment model of the lung is a simple description of a complex organ. The model's simplicity is both a strength and a weakness. By maintaining only two compartments and distributing NO production and consumption uniformly within the airway wall, the model needs only three unknown parameters (J'_{awNO}, D_{awNO}, and $C_{alv,ss}$) to completely specify NO exchange. This is clearly a strength as a more complex model introduces additional unknown parameters. However, the gross simplifications in the model have two important limitations. First, they limit the interpretation of the results to partitioning the relative

FIGURE 7—A. Percent change of C_{awNO} from baseline to 3, 30, and 120 min postexercise challenge in 10 healthy subjects. *Open* and *closed symbols* represent percent change of C_{awNO} in each individual, and *bar* indicates mean percent change. B. Individual levels of C_{awNO} with corresponding population mean (*horizontal bar*) from baseline to 3 min postexercise (base-3postEX), from baseline to 30 min postexercise (base-30postEX), and from baseline to 120 min postexercise (base-120postEX).

Our observations reflect the dynamic changes in NO exchange that occur postexercise in the acute phase (3 min postexercise) or relatively late phase (30 and 120 min postexercise). At first glance, our results for exhaled concentration appear to contrast with that of De Gouw et al. (7), who reported a decrease and an increase in exhalation concentration at 5 and 30 min postexercise in healthy adults. However, De Gouw et al. (7) reported mean changes in exhaled concentration at $\dot{V}_E = 100$ mL·s^{-1} of ~ -10% and ~ 20% from baseline at 5 and 30 min postexercise, respectively, which are relatively small

TABLE 3. Experimentally obtained and model predicted plateau NO concentration C_{NOplat} for baseline, 3, 30, and 120 min postexercise challenge. A. Experimentally obtained plateau NO concentration C_{NOplat}.

Subject	C_{NOplat} (~50 mL·s^{-1}) $\dot{V}_E$								C_{NOplat} (~250 mL·s^{-1}) $\dot{V}_E$							
	Base		3-PostEX		30-PostEX		120-PostEX		Base		3-PostEX		30-PostEX		120-PostEX	
	(ppb)	(mL·s^{-1})	(ppb)	(mL·s^{-1})	(ppb)	(mL·s^{-1})	(ppb)	(mL·s^{-1})	(ppb)	(mL·s^{-1})	(ppb)	(mL·s^{-1})	(ppb)	(mL·s^{-1})	(ppb)	(mL·s^{-1})
1	10.1	53.0	11.7	47.1	11.7	51.3	11.1	56.4	2.50	261	3.13	261	3.55	262	3.38	270
2	8.81	48.8	8.59	50.1	10.6	49.5	10.4	49.6	2.16	249	3.09	230	3.34	230	3.03	260
3	12.9	47.9	9.15	50.3	13.5	41.8	12.6	56.0	4.03	251	3.19	262	3.51	258	4.26	274
4	20.8	57.6	20.1	57.6	24.5	54.3	21.2	53.8	6.67	269	7.28	257	7.67	253	7.40	255
5	1.87	63.8	3.82	52.0	3.70	54.8	4.58	53.3	0.65	230	1.49	230	1.58	208	1.54	240
6	13.0	49.2	8.34	56.4	10.4	58.9	9.52	50.3	3.44	248	3.05	251	4.17	249	3.00	245
7	5.10	50.1	5.20	60.3	5.36	53.4	5.64	57.4	1.52	226	2.24	230	2.49	192	2.75	227
8	5.14	45.2	3.47	54.3	5.50	51.3	6.14	57.0	1.74	197	1.40	228	2.31	261	2.72	250
9	2.17	58.8	2.44	67.3	4.05	51.4	3.15	62.6	0.92	254	1.23	259	1.05	259	1.18	272
10	17.9	54.1	17.2	64.3	20.0	51.5	18.9	55.2	6.64	254	7.62	246	6.34	261	6.97	244
Mean	9.78	52.9	9.00	56.0	10.9	51.8	10.3	55.2	3.03	244	3.37	245	3.60	243	3.62	254
SD	6.43	5.78	5.90	6.54	6.95	4.40	5.98	3.75	2.17	20.9	2.28	14.4	2.05	25.0	2.07	15.4

Base, baseline; 3-postEX, 3 min postexercise challenge; 30-postEX, 30 min postexercise challenge; 120-postEX, 120 min postexercise challenge.

TABLE 3B. Model predicted plateau NO concentration C_{NOplat}.

Subject	C_{NOplat} (50 mL·s^{-1})				C_{NOplat} (250 mL·s^{-1})			
	Base	3-PostEX	30-PostEX	120-PostEX	Base	3-PostEX	30-PostEX	120-PostEX
1	10.2	10.4	11.3	12.9	2.77	3.41	3.17	3.84
2	8.50	8.51	11.2	10.9	2.59	3.09	3.22	3.72
3	13.0	10.6	11.0	13.3	4.60	3.73	3.24	4.63
4	19.9	20.68	24.6	20.8	7.04	7.81	8.40	8.68
5	3.73	3.64	4.07	5.07	1.20	1.11	1.26	1.53
6	13.1	8.82	11.8	9.33	4.76	2.91	4.29	2.84
7	5.94	5.60	5.60	6.25	1.56	2.17	2.39	2.63
8	4.02	3.84	5.90	7.22	1.53	1.39	2.44	2.68
9	2.75	3.17	4.02	5.01	0.93	1.22	1.39	1.67
10	17.9	17.2	19.8	18.8	7.62	7.80	7.87	8.13
Mean	9.91	9.25	10.9	11.0	3.46	3.46	3.77	4.04
SD	6.02	5.86	6.79	5.54	2.43	2.47	2.47	2.49

Base, baseline; 3-postEX, 3 min postexercise challenge; 30-postEX, 30 min postexercise challenge; 120-postEX, 120 min postexercise challenge.

contribution of the alveolar and airway regions toward exhaled NO, and the relative impact of diffusion-related (D_{awNO}) and metabolic-related (J'_{awNO}) factors on the airway contribution. Second, the simplifications may impact the interpretation of the results.

There are three main simplifications in the two-compartment model that could impact values of the flow-independent parameters. The first is the assumption that airway NO flux is uniformly distributed per unit volume in the airway tree. Experimental evidence suggests that the larger airways may be a more prominent source relative to smaller airways (8,25). This may reduce the volume of NO that accumulates in the airway compartment during breathhold as the concentration difference between the airway wall and the gas phase would decrease at a faster rate. The model would then underestimate the average airway wall flux for the entire airway tree. The second major simplification is the absence of axial or longitudinal diffusion of NO in the gas phase as mechanism of transport. We and others have recently demonstrated theoretically that axial diffusion may be a significant mechanism of NO transport from the airways into the alveolar region, thus acting as a sink for NO (22,36). The result may be an underestimation in the airway wall flux and thus production rate. Although both of these simplifications may impact the absolute values of the flow-independent NO parameters, neither is likely to impact the trend observed after high-intensity exercise and thus the major conclusions of this study. The final

major assumption is that of a constant alveolar concentration during exhalation. At a constant exhalation flow rate, the exhaled concentration of NO reaches a nearly constant value, suggesting that the alveolar and airway contributions are constant. We have demonstrated experimentally that the diffusing capacity of NO in the alveolar region is a positive function of lung volume (30,35). However, the impact on the exhalation profile is likely offset by changes in the alveolar production rate (31) such that the alveolar concentration is nearly constant for exhalation times greater than 10 s (11,32).

In summary, we have quantified several flow-independent parameters characteristic of NO exchange in response to high-intensity exercise in healthy controls. Significant changes were observed in J'_{awNO}, D_{awNO}, and C_{awNO} 3 min postexercise challenge, despite no significant changes in exhaled concentration (C_{NOplat}). Thus, the flow-independent NO parameters provide greater specificity in characterizing NO exchange. We conclude that exercise acutely enhances elimination of NO from airway tissues stores. This effect may be due to enhanced ventilation or an enhanced ability of NO to diffuse from the airway tissue to the gas phase. The latter suggests endogenously produced NO may be useful to probe metabolic and structural features of the airways.

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