

# Nitric Oxide Accumulation in the Nonventilated Nasal Cavity

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## ABSTRACT

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**Background** Nasal nitric oxide is present in high concentrations in the upper airway relative to the lower respiratory tract.

**Objective** To explore the rate of nitric oxide accumulation in the nonventilated nasal cavity.

**Methods** In 9 healthy subjects previously trained to close the soft palate, steady-state plateau nitric oxide levels were recorded while air was aspirated through the nasal airway in series at a constant flow rate. Nitric oxide was then allowed to accumulate in the nasal cavity by occluding both nares and keeping the velum closed. After varying occlusion times, peak nitric oxide levels and a second plateau were ascertained.

**Results** While the subjects aspirated air at a constant flow, there was a slow rise to a first nitric oxide plateau. On opening to the analyzer after the accumulation period, the peak nitric oxide level was several times higher than the initial plateau (range, 2810-19,008 ppb) and then slowly returned to previous plateau levels. There was no significant difference between initial and second plateau nitric oxide levels for any period. The accumulated nitric oxide peak increased in direct proportion to the accumulation time ( $P < .001$ ).

**Conclusions** Nitric oxide concentrations accumulate in the nonventilated nasal cavity in proportion to the time of nonventilation. Peak nasal nitric oxide values after accumulation are similar to published sinus nitric oxide measurements obtained by direct puncture. These results suggest an important alternative source of nitric oxide in humans.

NITRIC OXIDE (NO) is present in the human airway,<sup>1</sup> and high nasal concentrations of nitric oxide were first detected by Alving et al.<sup>2</sup> The source of high nitric oxide levels in the nose is still uncertain, but there is evidence that the upper airways (ie, paranasal sinuses, nasal cavities, or nasopharynx) could be the main source of this gas in exhaled air.<sup>3,4</sup>

Nasal nitric oxide may subserve physiological functions in the upper airway. This chemical may represent the very first line of defense in the airways, possibly acting on pathogens even before they reach the nasal mucosa.<sup>3</sup> Its concentration in upper airways is several times higher than that required for bacteriostasis<sup>5</sup> and antiviral effect.<sup>6</sup> It also acts as a mediator of mucociliary activity<sup>7</sup> and mucus secretion.<sup>8</sup> Autoinhalation may also provide another endogenous function for nitric oxide, regulating the ventilation-perfusion ratio.<sup>9</sup> Nitric oxide has also been shown to play a role in neurally mediated bronchodilation<sup>10</sup> and in the modulation of airway reactivity.<sup>11</sup>

Nitric oxide may also be involved in the pathophysiology of several diseases. High nitric oxide levels are found in patients with noninfectious inflammations, such as allergic rhinitis.<sup>12</sup> Conversely, nasal nitric oxide levels are substantially diminished during active bacterial sinusitis<sup>13</sup> and in cystic fibrosis<sup>14</sup> and primary ciliary dysfunction (Kartagener syndrome).<sup>15</sup>

We were interested in measuring the concentration of accumulated nitric oxide in the isolated nasal cavity, since the dynamics of nitric oxide accumulation may have important implications in the determination of how nitric oxide plays its physiological roles. In this study, we evaluated the rate and the level of accumulation of nitric oxide in the nonventilated nasal cavity to see if nitric oxide levels were as high as those obtained by direct puncture of the sinuses.<sup>3</sup>

## MATERIAL AND METHODS

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### SUBJECTS

Nine nonsmoking, healthy subjects were selected from laboratory personnel. We excluded subjects with acute or chronic nasal pathologic conditions, upper respiratory infection 4 weeks prior to the study, use of corticosteroids within 6 weeks of the study, current smoking, and any significant medical conditions.

### NITRIC OXIDE ANALYSIS

A rapid-response chemiluminescent nitric oxide analyzer (Sievers NOA 280; Sievers Instruments Inc, Boulder, Colo) was employed. A daily 2-point calibration was performed with an analyzed standard gas (1.6 ppm). The analyzer sample rate was 3.3 mL/s for all measurements. The lower detection limit for nitric oxide was 1 to 2 ppb. Nitric oxide levels in ambient air were recorded before and after each subject was studied.

### DATA ACQUISITION

The analog output was fed to a data acquisition program (DasyLab for Windows; DasyTec Corp, Amherst, NH) with a real-time monitor display of nitric oxide vs time, and data were saved via an analog-digital converter to the computer hard disk. Nitric oxide concentrations were calculated using a data analysis program (Microsoft Visual Basic; Microsoft Corp, Redmond, Wash).

### METHOD

#### Initial Aspiration: First Plateau

Subjects rested for 15 minutes before measurements and all the tests were performed between 8 and 10 AM. The volunteers were trained to breathe tidally through the mouth while voluntarily closing the soft palate. Hence, the nasal airways were isolated from the lower respiratory tract. Velum closure was confirmed by a nitric oxide trace that rose to an initial steady plateau as opposed to tidal oscillations if the velum remained open.

A tapered tube (length, approximately 4 cm; internal diameter, 1 cm) was inserted into one nostril, ensuring that no leaks existed. The opposite nostril remained open to the room air. Thus, the room air was aspirated through the nasal airway in series at a constant sampling flow (3.3 mL/s).

The nitric oxide analyzer sample line was attached via a 3-way stopcock to the nostril tube.

The nitric oxide profile rose to a plateau 20 to 40 seconds after the beginning of the maneuver.

#### Nonventilation of Nasal Cavity

Once the first plateau was achieved, the analyzer was switched out of the circuit, the air inlet tube was clamped for fixed periods (15, 30, 60, and 120 seconds), and the opposite nostril was blocked by the subject's thumb to prevent nitric oxide leaking out. Subjects continued to breathe through the mouth with the velum closed. The procedure was repeated for each nitric oxide accumulation time.

#### Measurement of Accumulated Nitric Oxide: Second Plateau

After the required period, the stopcock was opened and the analyzer was switched back to the nasal tube. Meanwhile, the opposite nostril was reopened. The nitric oxide peak concentration, representing the accumulated nitric oxide, and the subsequent plateau were recorded. Three separate measurements were recorded for each accumulation period.

The initial plateau, peak, and second plateau were compared, and the effect of length of the accumulation period on nasal nitric oxide concentrations was noted.

### STATISTICS

The nitric oxide accumulation experiment results were analyzed by nonparametric methods (Kruskal-Wallis), once for time effect and once for nitric oxide levels. A regression model was fitted for the nitric oxide peak by increasing time.

## RESULTS

The data distribution was not normal. Nine subjects were studied (6 men and 3 women; age range [median], 21-53 [46] years). Four of the 9 subjects found it initially difficult to keep the velum closed for the longer periods needed in this experiment, as evidenced by initial tidal oscillations in the nitric oxide trace. However, after training, all of them performed the maneuvers as required.

The peak nitric oxide concentrations for the 4 accumulation periods for each subject are shown in [Table 1](#). There was a slow rise in nitric oxide concentration to an initial plateau (Q1-Q3 range [median], 1623-2126 [1816] ppb). When the analyzer sampling line to the nostril tube was opened after the accumulation period, there was a rapid rise to a peak nitric oxide level that was several times higher than the first plateau (Q1-Q3 range [median], 5447-8289 [6750] ppb), reaching in some maneuvers as high as 19,000 ppb. Then a slow return to a second plateau was registered (Q1-Q3 range [median], 1968-2384 [2171] ppb) ([Figure 1](#)).

Nitric Oxide Peaks According to the Accumulation Period\*

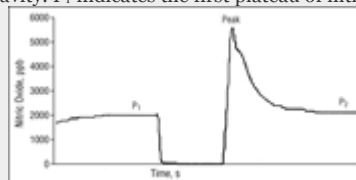
Patient No.	Nitric Oxide Levels, ppb			
	15 s	30 s	60 s	120 s
1	4177.7	5454.0	7451.0	9782.0
2	4062.0	5454.3	8662.0	7354.3
3	4155.7	5582.3	5756.7	11737.3
4	4061.6	4991.3	8046.7	11960.7
5	5121.6	7760.3	10669.7	14107.0
6	6838.3	9284.3	12560.0	17788.7
7	4622.5	4773.0	5903.0	7466.0
8	5777.3	6789.0	9789.3	19008.7
9	2816.3	4048.0	5137.5	7300.5
Median	4177.7	5454.3	8046.7	11737.3
Q1	4062.0	4991.3	5903.0	7466.0
Q3	5121.6	6789.0	9789.3	14107.0

\*Q1 indicates first quartile (25th percentile); Q3, third quartile (75th percentile).

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**Figure 1.**

Nitric oxide profile in the nonventilated nasal cavity. P<sub>1</sub> indicates the first plateau of nitric oxide concentrations; P<sub>2</sub>, second plateau.

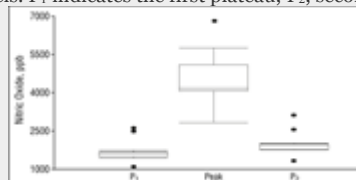


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The first and second plateaus were not significantly different for any maneuvers ([Figure 2](#)). The peak nitric oxide value was directly related to the duration of the accumulation period (nonventilated time) ([Figure 3](#)). The correlation between the peak nitric oxide concentration and the period of accumulation was  $r^2=0.72$  ( $P<.001$ ).

**Figure 2.**

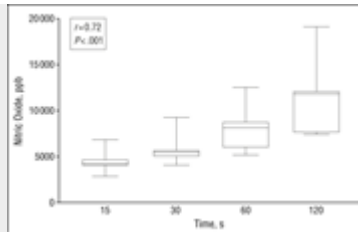
Peak and plateau median nasal nitric oxide levels. P<sub>1</sub> indicates the first plateau; P<sub>2</sub>, second plateau. Bars indicate SEs.



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**Figure 3.**

Relationship between peak exhaled nitric oxide level and the period of nonventilation of the nasal cavity. Bars indicate SEs.



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A regression model was fitted for the nitric oxide peak by increasing time. Natural logarithms of nitric oxide measurements were used. The nitric oxide values were not normally distributed by increasing time (test for normality,  $P < .001$ ), and the variances varied widely (Bartlett  $P = .002$ ). Transformation on a normal logarithm scale corrected this (test for normality,  $P = .51$ ; Bartlett  $P = .68$ ); hence, transformed values were used. The equation was  $\text{NO} = \exp(8.3822 + 0.008219t)$ , in parts per billion, where  $t$  is the time of nonventilation.

## COMMENT

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We noninvasively detected nitric oxide levels in the parts per million range in the nonventilated nasal cavity, levels of the same order of magnitude as those described by Lundberg<sup>3</sup> in the nasal sinuses by direct sinus aspiration. The median nasal nitric oxide value after 2 minutes of accumulation was approximately 12 ppm (range, 7.3-19 ppm). These high concentrations are several orders of magnitude greater than those measured in the lower respiratory tract.

Nitric oxide accumulates physiologically in periods of nonventilation of the nasal cavity; for example, during nasal cycle, speech, or swallowing or in mouth breathers. The subsequent resumption of nasal breathing will then result in the inhalation of nitric oxide to the lower airways and lungs. This may have physiological effects in the lower airways, such as regulation of the ventilation-perfusion relationship,<sup>8</sup> or it may play a role in host defense.<sup>3,5,7,8</sup> The high nitric oxide levels reached in these nonventilated periods may also be viewed as part of the normal upper airway defenses, causing bacteriostasis, viristasis, and modulating ciliary motility. The reduction or lack of ventilation in the nares in some clinical situations will also promote nasal nitric oxide accumulation.<sup>12</sup>

The relative contribution of the nasal cavity and the paranasal sinuses to the nitric oxide levels cannot be ascertained from this study. The upper airway is a complex system of communicating cavities. Thus, the nitric oxide accumulating in the nasal cavity could come from an adjoining chamber, such as the paranasal sinuses, the nasal cavities, or the nasopharynx, as the epithelium of these cavities has been shown to express nitric oxide synthase.<sup>16-18</sup>

It is our contention, however, that most of the accumulated nitric oxide in the nonventilated nasal cavity registered in this study came from the nasal mucosa. First, the rate of nitric oxide increase observed was much more rapid than would be expected from the previously quantified diffusion rate of several gases in and out of the sinuses.<sup>19</sup> Gas transfer between nose and sinuses is dependent on several factors, such as functional size of the ostium, nasal air flow, nasal respiratory pressure, volume of the sinus, size and shape of the nasal cavity, and composition of the gas mixture and its absorption by the mucosa.<sup>19-21</sup> Most sinus ostia are small and are located deep in the middle meatus; thus, ventilation is restricted, especially at low flows. Considering these factors and the short period used in this experiment (up to 2 minutes), we would not have expected that sinus nitric oxide could have leaked out sufficiently to explain such high values.<sup>21</sup> Second, a previous study in the same laboratory has shown in 1 subject that nasal nitric oxide concentration accumulated to 29 ppm after 5 minutes of nonventilation while the maxillary sinus ostia were plugged with wet cotton wool.<sup>22</sup> Consequently, even while preventing gas diffusion from the major sinuses to the nasal cavity, high nasal nitric oxide concentrations were achieved. In that study, only approximately 12% of the nasal nitric oxide came from the sinuses. Third, the reported decrease in nasal nitric oxide concentration in the presence of xylometazoline,<sup>23</sup> which should ensure enhanced diffusion of nitric oxide from the sinuses, is another argument against the sinuses being the predominant site of nitric oxide production. Other authors have reported that nitric oxide is also produced in the nasal epithelium.<sup>17,24</sup>

DuBois et al<sup>24</sup> argue that the amount of nitric oxide measured in the nose reflects not the gas production, but the nitric oxide release (ie, the balance of nitric oxide production and absorption and its physical removal by ventilation). Thus, the rate of nitric oxide rise demonstrated in our study was probably a balance between production, absorption, and removal by the sampling pump. However, during the plateau phase, the production fraction equals the absorption fraction plus the part removed by sampling ([Figure 1](#)).

Although the volumes of the nasal cavity and sinuses are similar (both approximately 15-20 cm<sup>3</sup>), the nasal cavity has a larger surface area (approximately 300 cm<sup>2</sup>) than the sinuses (maxillary sinus surface, 30 cm<sup>2</sup>).<sup>25</sup> As we detected nitric oxide concentrations in the nasal cavity during periods of absence of airflow similar to levels reported in the sinuses,<sup>3</sup> it is possible that the nitric oxide production in the nasal cavity per square centimeter is lower than in the sinuses. This may be explained in part by different levels of nitric oxide production by the distinct nitric oxide synthases in the nasal mucosa and paranasal sinuses.<sup>16</sup> Lundberg et al<sup>16</sup> have shown that nitric oxide synthase activity in healthy sinus mucosa is mainly due to activity of Ca<sup>2+</sup>-independent nitric oxide synthase, a different kind of enzyme previously demonstrated in nasal mucosa.<sup>18</sup>

The high nitric oxide levels detected in the nonventilated nasal cavity and their possible origin in the nasal mucosa are comparable to the levels of paranasal sinus nitric oxide production suggested by Lundberg.<sup>3</sup> The high levels of nitric oxide suggest, however, the possibility of an important alternative source of nitric oxide in humans.

## CONCLUSIONS

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Nitric oxide concentrations accumulate rapidly in the nonventilated nasal cavity. These peak values are similar to the levels described after aspiration of the paranasal sinuses.

Nitric oxide concentrations in the nonventilated nasal cavity increase with time in a predictable pattern.

Further studies are necessary to determine the relative contribution of the sinuses and nasal epithelium to accumulated nasal nitric oxide. However, the results reinforce the probability that the nasal mucosa is an important site of nitric oxide production.

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