Inhaled nasopharyngeal nitric oxide concentrations during unilateral nostril breathing – a pilot study

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Research

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Abstract

Objective: To assess the influence unilateral nostril breathing has on mean inhaled nasopharyngeal nitric oxide (NO) concentrations compared with unobstructed bilateral nostril breathing in individuals demonstrating a nasal cycle.

Methods: After determining the patent and congested nasal sides in healthy adult volunteers (N=10), and sampling air at both nostrils, a small diameter gas sampling tube was passed along the floor of the nose into the volunteer's patent nostril, until it was stationed in the nasopharynx. Nasopharyngeal NO concentrations were then assessed during normal nasal at-rest tidal breathing during three different nasal breathing states: first both nostrils, then allocated in randomised order, patent side only, and congested side only.

Results: Nasopharyngeal NO concentrations were consistently higher on both exhalation and inhalation during unilateral congested side nostril breathing, when compared with unilateral patent side nostril breathing, and breathing through both nostrils.

Conclusions: During unilateral nostril breathing, inhaled nasopharyngeal NO concentrations are consistently higher on the congested side of the nose.

Introduction

The nasal cycle, where periodic changes in nasal airway geometry occur, results in a greater tidal nasal air-flow volume passing through one side of the nose: the patent airway, while a lesser volume passes through the other side: the congested airway [1, 2]. The purpose(s) of the nasal cycle continues to be debated and discussed [3]. The nose has an important role in maintaining airway health by entrapping inhaled pathogens, allergens and pollutants, as well as heating and humidifying inhaled air [4]. The nasal cycle through fluctuation in airflow partitioning between each airway is also thought to enable the upper airway to accommodate the contrasting roles of air conditioning and the removal of entrapped contaminants [5].

Nitric oxide (NO) is a vasodilator and neurotransmitter gas produced by NO synthase from arginine and oxygen [6]. The nasal airways, particularly the paranasal sinuses, produce NO, which has antibacterial and antiviral actions [7], and also plays an important role in modulating epithelial function including ciliary beat frequency [7–9]. Previous research has shown that nasal NO concentrations are nasal airflow dependent [8, 10]. The increased inhaled NO concentration in the congested side of the nose associated with the nasal cycle, is thought related to reduced airflow [8]. Although NO concentrations on each side of the nose might change with patency status, the NO concentrations delivered to the lower airways remain relatively stable, and reduce with increasing age, poor diet and inactivity [11]. Previous research has investigated nasal NO concentrations in a non-ventilated nose [10], as well as nasal NO concentrations associated with the nasal cycle [8]. These studies found nasal NO concentration is nasal airflow dependent, and pharyngeal NO concentrations correlated negatively to nasal volume.

Our investigation sought to confirm the influence unilateral nostril breathing (UNB) has on mean inhaled nasopharyngeal peak NO concentrations compared to unobstructed bilateral nostril breathing (BNB), and if this was the case, identify the nasal cycle phase associated with any increase in inhaled nasal NO concentration.

Methods

We conducted a clinical study at the Auckland University of Technology BioDesign Lab between April and July 2019. Ten healthy volunteers (age range, 20 to 63 years; mean $\pm$ standard deviation, 43.1 $\pm$ 18.5 years; males 8, females 2) were recruited for this study. Exclusion criteria were a history of allergic rhinitis, sinusitis, or previous nasal surgery. All volunteers underwent a nasal endoscopic examination by an otolaryngologist. Volunteers with a significant septal deviation, turbinate abnormalities or inflammation were excluded. The volunteer was also excluded if the gas sampling tube was unable to pass comfortably along the floor of the volunteer's nasal cavity to the nasopharynx.

Prior to and after gas testing, the nostril patency status was determined by using two spirometers adapted to measure individual nostril airflow. Each volunteer was asked to strongly exhale via the nose, while holding a spirometer against each nostril (Fig. 1). Peak exhaled airflow measurement for each side of the nose was then compared, with the side demonstrating the greatest airflow being designated 'patent' and the side showing a lower value labelled as 'congested'.

NO concentrations were measured by a rapid response chemiluminescence NO analyser (Logan Research Limited LR2149 series NO gas analyser, UK), calibrated prior to every participant test using a calibration gas mixture (200 ppb, BOC). A small diameter (0.79 mm internal diameter x 1.59 mm external diameter) polytetrafluoroethylene (PTFE - Teflon) gas sampling tube (Cole-Palmer, C-P06407-41), was connected at one end to the NO analyser via a hydrophobic disc filter element (PTFE 1 micron filter). The other loose end of the Teflon gas sampling tube had two additional small side holes to prevent the tube from sucking against the walls of the nasal airway. All gas sampling was undertaken with a sampling flow rate of 0.25 litres/min for a period of 40 seconds using a data sampling rate of 40 milliseconds.

After identifying the patent side of the nose using the nasal spirometer test, the NO concentration at the nasal vestibule was measured. This entailed the gas sampling tube being held at the left nostril (without occluding the right nostril) and the volunteer was asked to breathe 'normally', while the tidal nasal airflow was sampled over a 40 second period. This procedure was then repeated with the gas sampling tube held at the right nostril.

The gas sampling tube was then passed into the volunteer's patent nostril, along the floor of their nose until it was stationed in the nasopharynx. This was confirmed by the participant experiencing the sensation of the tube abutting against the nasopharynx wall and visual confirmation of the graduated tube length. Once the participant was comfortable, nasopharynx NO concentration measurement was undertaken. This entailed first measuring during BNB, before undertaking UNB repeated for both sides of the nose, with the order decided by coin toss.

On conclusion of testing, the measuring tube was removed from the nose and the state of the nasal cycle again determined using the spirometers.

Acquired data was transferred in .txt format from the gas analyser to a PC for analysis using an external USB hard drive. Data analysis was split into the different breathing conditions in the measurement and undertaken using MatLab script (MathWorks, USA). After identifying all gas concentration inhalation peaks in the datasets, their mean was then calculated for each of the three different breathing conditions. Descriptive statistics were then applied to the relative data grouped by breathing condition using IBM SPSS version 24. In SPSS, a Shapiro-Wilk parametric test was used to test if the data is normally distributed [12].

Results

All the breathing condition gas concentration means were found to be normally distributed.

Using a double-sided paired t-tests for all statistical analysis, there was no significant difference in NO concentrations detected at the nasal vestibule between the individual's patent and congested nostrils during either exhalation ($p = 0.3$) or inhalation ($p = 0.9$) breath phases (Table 1). Comparing the increase in exhaled NO concentration from the ambient inhaled level also showed no significant difference between either nostril. The mean exhaled NO concentration during BNB was 17.9 ppb (SD = 0.4).

During BNB, mean nasopharyngeal NO concentrations were significantly higher on inspiration compared to expiration ($p = 0.04$) (Table 2). The contribution of inhaled NO sourced from the nose was calculated from the difference between the mean ambient inhaled congested nostril NO level (Table 1) and mean inhaled nasopharynx concentrations (Table 2).

During UNB breathing, mean inhaled nasopharyngeal NO concentrations were significantly higher than exhaled values for all participants during patent-side UNB ($p = 0.003$) and congested-side UNB ($p = 0.04$). The mean inhaled nasopharyngeal NO concentrations were significantly higher ($p = 0.01$) when breathing exclusively through the congested nostril compared to the patent nostril (Table 3).

Figure 2 presents a summary comparison of mean inhaled nasopharyngeal NO peak concentrations for all three breathing states with whiskers showing the standard error of the calculated means. The data is grouped per volunteer and divided by the different breathing conditions. The dark blue coloured bars display the mean inhaled nasopharyngeal NO concentrations during BNB. The orange coloured bars show these levels during breathing UNB through the congested side of the nose and the yellow coloured bars show these values during UNB exclusively through the congested side of the nose.

On average, the ten participants experienced an eight-fold mean increase in inhaled NO concentration over unobstructed nasal breathing occurred during UNB congested-side breathing. In contrast, UNB patent-side breathing resulted in a 40% average reduction in mean inhaled NO when compared to

unobstructed BNB. Table 4 presents the percentage change in peak inhaled NO concentration at the nasopharynx during UNB for all participants.

When the status of the participant's nasal cycle was checked at the end of the experiment only participant 9 demonstrated a change (Table 5).

Discussion

Mean inhaled ambient NO concentrations at the nasal vestibule (Table 1) were low for all participants, and typical of that found indoors [13]. Exhaled nasal NO concentrations, which are normally used to diagnose airway inflammation [14] (Tables 1 and 2) were in the healthy range [15], indicating none of our participants had underlying airway inflammatory issues. For each participant in the current study, minor variation in mean ambient NO levels measured at the vestibule when comparing the patent and congested sides of the nose (Table 1) was seen. While the reasons for this are unclear, it is most likely due to poor sensor resolution caused by the low NO concentrations being recorded. For our analysis, the difference between mean inhaled nasopharyngeal and mean ambient inhaled congested nostril NO concentrations were compared (Tables 1 and 2), to determine the mean NO contribution provided by the nose during BNB inhalation. This work (Table 2) confirms previous studies showing that the nose is a major contributor of inhaled NO [16–18]. Variation in inhaled NO concentrations between participants during BNB could be caused by many factors. Diet, exercise [19], and aging [20] are all thought to influence NO availability. When concentrations of exhaled NO during either congested-side or patent-side UNB were compared (Table 3), exhaled NO values followed that of the inhaled values, albeit at a lower level. It is possible that the higher exhaled NO concentrations were sourced from residual NO in the anatomical dead space. There was also no significant difference in exhaled NO concentrations between either side of the nose.

The current study aimed to measure inter-nasal inhaled NO concentration relative to nasal cycle status. Mean nasopharyngeal NO concentrations, as a marker of NO availability from within the nose, were extremely high during congested-side UNB, compared to either patent-side UNB or BNB (Table 3 and Fig. 2). This finding supports the earlier findings by Chatkin et al. [10] and Qian et al. [8], who suggested the nasal cycle permitted local accumulation of NO in the congested side of the nose. An earlier investigation measuring oropharyngeal NO concentrations had used the nasal decongestant (xylometazoline) prior to occluding one nostril with cotton wool, which is not representative of nasal physiological conditions [8]. Our study assessed nasopharyngeal NO concentrations during BNB and UNB under more appropriate physiological conditions where the small size of the measuring tube (1.59 mm in external diameter) and the passage of the tube along the floor of the patent nostril, should not have dramatically altered nasal resistance.

No recognised normal reference range for nasal NO (nNO) exists [9]. A normal variation in nNO concentrations of 20–25% has been reported [21]. nNO concentrations can be influenced by many external factors such as season, time of day, exercise, breathing method and the type of analyser used [9,

22]. Our NO concentrations on exhalation (Table 1) are consistent with previously reported values [23], however higher values have also been reported [9]. Previous NO concentrations in the oropharynx had previously been assessed at approximately 40 ppb [8], which is consistent with our nasopharyngeal NO recordings (Table 2).

Nasal epithelial cells can synthesize NO by using ambient O₂ as a substrate [24]. After a period of time, NO production might cease, if insufficient O₂ from the surrounding air is available. The nasal cycle could allow the patent side of the nose to contribute inhaled NO to the lungs, while NO concentrations on the congested side of the nose are replenished. When the NO concentrations are replenished on the congested side, this side might then become the patent side. This would be in keeping with the theory that the nasal cycle enables the epithelial cells and glands to rest and recharge [25] as well as enabling the upper airway to accommodate the contrasting roles of air conditioning and the removal of entrapped contaminants [5]. The higher NO concentrations on the congested side would facilitate mucociliary transport and provide increased antimicrobial action [8]. The nasopharyngeal mixing results in a steady NO being delivered to the lungs. High NO concentrations have an antibacterial cleansing action [26], but high NO concentrations may also contribute to lung inflammation [27]. Participant 9, whose “patent airway” changed sides during testing (Table 5), demonstrated the lowest increase (+ 4%) in inhaled NO during congested-side UNB. While not an objective of this study, this single result supports the earlier finding by Chatkin et al. that found nasal NO accumulation is time dependant [10] and suggests that variation in NO concentrations recorded by our study could all have been influenced by the time each individual had experienced a steady-state phase of their nasal cycle. Any further nasal NO studies should possibly consider taking long-term nasal cycle measurement prior to gas concentrations being measured given the temporal variability of nasal NO concentrations. While an earlier study has shown that nasal NO concentration is dependent on nasal airflow and nasal cycle status [8], our study has demonstrated that augmentation of inhaled NO concentrations is possible during congested-side UNB. Further research with more careful monitoring of the nasal cycle and repeated nasopharyngeal recordings over longer time periods would clarify how NO concentrations fluctuate between the congested and patent sides of the nose during both the steady-state breathing and switching phases of the nasal cycle.

Conclusions

Inhaled nasopharyngeal NO concentrations appear to change with the nasal patency status associated with the nasal cycle. For a nose exhibiting a nasal cycle, inhaled nasopharyngeal NO concentrations are consistently higher during congested-side UNB.

Abbreviations

BNB
bilateral nostril breathing
NO
nitric oxide

UNB
unilateral nostril breathing

Declarations

Ethics approval and consent to participate

Ethics approved obtained through the Auckland University of Technology Ethics Committee (AUTEC approval 17/135).

Consent for publication

All participants have provided consent to publish.

Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available due participant confidentiality but are available from the corresponding author on reasonable request.

Competing interests

DEW and JB are listed inventors of a lateralised nasal breathing device.

Funding

Not applicable.

Authors' contributions

DEW and JB designed the study, THAS undertook data collection, THAS, JB, MK and DEW were involved with manuscript preparation. All authors read and approved the final manuscript.

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References

1. Eccles R. Nasal airflow in health and disease. *Acta Otolaryngol.* 2000;120:580–95.
2. Hanif J, Jawad S, Eccles R. The nasal cycle in health and disease. *Clin Otol Allied Sci.* 2000;25:461–7.

3. Pendolino AL, Lund VJ, Nardello E, Ottaviano G. The nasal cycle: a comprehensive review. *Rhinology Online*. 2018;1:67–76.
4. Elad D, Wolf M, Keck T. Air-conditioning in the human nasal cavity. *Respir Physiol Neurobiol*. 2008;163:121–7.
5. White DE, Bartley J, Nates RJ. Model demonstrates functional purpose of the nasal cycle. *Biomed Eng Online*. 2015;14:38.
6. Young D. Nitric oxide. *BJA CEPD Reviews*. 2002;2:161–4.
7. Lundberg JO. Nitric oxide and the paranasal sinuses. *Anat Rec (Hoboken)*. 2008;291:1479–84.
8. Qian W, Sabo R, Ohm M, Haight JSJ, Fenton RS. Nasal nitric oxide and the nasal cycle. *Laryngoscope*. 2001;111:1603–7.
9. Ren L, Zhang W, Zhang Y, Zhang L. Nasal nitric oxide is correlated with nasal patency and nasal symptoms. *Allergy Asthma Immunol Res*. 2019;11:367–80.
10. Chatkin JM, Qian W, McClean PA, Zamel N, Haight J, Silkoff P. Nitric oxide accumulation in the nonventilated nasal cavity. *Arch Otolaryngol Head Neck Surg*. 1999;125:682–5.
11. Torregrossa AC, Aranke M, Bryan NS. Nitric oxide and geriatrics: Implications in diagnostics and treatment of the elderly. *Journal of geriatric cardiology: JGC*. 2011;8:230–42.
12. Field A. *Discovering statistics using SPSS. Sage publications* 2009.
13. Jarvis DJ, Adamkiewicz G, Heroux M-E, Kelly FJ. Nitrogen dioxide. In: *WHO Guidelines for Indoor Air Quality: Selected Pollutants*. Geneva: World Health Organization; 2010.
14. Maniscalco M, Sofia M, Pelaia G. Nitric oxide in upper airways inflammatory diseases. *Inflamm Res*. 2007;56:58–69.
15. Hogman M, Drca N, Ehrstedt C, Meriläinen P. Exhaled nitric oxide partitioned into alveolar, lower airways and nasal contributions. *Respir Med*. 2000;94:985–91.
16. Corbelli R, Hammer J. Measurement of nasal nitric oxide. *Paediatr Respir Rev*. 2007;8:269–72.
17. Lundberg JON, Weitzberg E. Nasal nitric oxide in man. *Thorax*. 1999;54:947–52.
18. Holden WE, Sippel JM, Nelson B, Giraud GD. Greater nasal nitric oxide output during inhalation: effects on air temperature and water content. *Respir Physiol Neurobiol*. 2009;165:22–7.
19. Roberts CK, Vaziri ND, Barnard RJ. Effect of Diet and Exercise Intervention on Blood Pressure, Insulin, Oxidative Stress, and Nitric Oxide Availability. *Circulation*. 2002;106:2530–2.
20. Sverdllov AL, Ngo DTM, Chan WPA, Chirkov YY, Horowitz JD. Aging of the Nitric Oxide System: Are We as Old as Our NO? *Journal of the American Heart Association*. 2014;3:e000973.
21. Bartley J, Fergusson W, Moody A, Wells AU, Kolbe J. Normal adult values, diurnal variation, and repeatability of nasal nitric oxide measurement. *Am J Rhinol*. 1999;13:401–5.
22. De Winter-de Groot KM, Van der Ent C. K.. Measurement of nasal nitric oxide: evaluation of six different sampling methods. *Eur J Clin Invest*. 2009;1:7207.
23. Leng G, Li Z, Wang Q. Detection of exhaled nitric oxide of healthy in Nanjing. *Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi*. 2012;26:769–71.

24. Nakano H, Ide H, Ogasa T, Osanai S, Imada M, Nonaka S, Kikuchi K, Iwamoto J. Ambient oxygen regulates epithelial metabolism and nitric oxide production in the human nose. *J Appl Physiol* (1985). 2002;93:189–94.
25. Eccles R. Neurological and pharmacological considerations. *In: Proctor DF, Andersen I, editors The nose: upper airway physiology and the atmospheric environment* 1982, Amsterdam: Elsevier Biomedical Press:191–214.
26. Lundberg JO, Farkas-Szallasi T, Weitzberg E, Rinder J, Lidholm J, Anggård A, Hökfelt T, Lundberg JM, Alving K. High nitric oxide production in human paranasal sinuses. *Nat Med*. 1995;1:370–3.
27. Van der Vliet A, Eiserich JP, Cross CE. Nitric oxide: a pro-inflammatory mediator in lung disease? *Respir Res*. 2000;1:67–72.

Tables

Table 1: Anterior mean nitric oxide concentrations (ppb) at the vestibule for the patent and congested nostrils during both inhalation and exhalation.

Volunteer	Mean of peak NO levels at vestibule (ppb)			
	Exhaled patent nostril	Ambient inhaled patent nostril	Exhaled congested nostril	Ambient inhaled congested nostril
1	11.4	0.8	13.4	2.7
2	25.9	19.4	27.7	17.2
3	7.9	3.9	7.3	2.9
4	17.5	8.5	14.5	4.6
5	37.5	30.4	38.1	28.8
6	24.3	16.6	22.3	14.1
7	45.0	31.0	40.6	26.7
8	22.2	10.5	82.6	19.1
9	6.6	1.0	30.4	11.8
10	15.4	5.8	8.1	1.1

Table 2: Mean nasopharyngeal nitric oxide concentrations (ppb) during expiration and inspiration through both nares while bilateral nostril breathing (BNB).

Peak nasopharyngeal NO levels during bilateral nostril breathing (ppb)			
Volunteer	Mean Exhaled	Mean Inhaled	Mean Nasal NO contribution
1	24.2	44.4	41.7
2	12.4	33.5	18.3
3	5.1	12.5	9.6
4	7.4	19.2	14.6
5	22.7	32.7	3.9
6	16.1	26.1	12.0
7	23.2	37.1	10.4
8	33.2	94.4	93.2
9	21.6	42.1	30.3
10	13.8	24.6	23.5

Table 3: Mean nasopharyngeal NO concentrations (ppb) for inspiration and expiration during exclusive patent-side and congested-side unilateral nostril breathing (UNB).

Participant	UNB – patent side		UNB – congested side	
	Nasopharyngeal mean exhaled NO level (ppb)	Nasopharyngeal mean inhaled NO level (ppb)	Nasopharyngeal mean exhaled NO level (ppb)	Nasopharyngeal mean inhaled NO level (ppb)
1	1.8	5.8	98.2	217.7
2	13.7	24.2	202.2	418.0
3	1.9	10.2	16.4	42.2
4	6.2	14.7	73.6	263.3
5	26.0	36.8	141.2	232.3
6	13.6	24.3	117.7	224.4
7	15.0	43.6	37.4	54.1
8	20.0	31.0	117.0	241.9
9	6.8	22.1	29.6	43.6
10	7.0	16.3	194.7	900.7

Table 4: Percentage change in mean nasopharyngeal NO concentrations (ppb) comparing UNB patent side and UNB congested side nasal breathing to bilateral nostril breathing (BNB).

Percentage change in peak inhaled NO nasopharyngeal concentrations comparing bilateral nostril breathing to unilateral nostril breathing		
Participant	UNB – patent side breathing (%)	UNB – congested side breathing (%)
1	-87	+391
2	-28	+1147
3	-19	+238
4	-23	+1268
5	13	+611
6	-7	+760
7	18	+46
8	-67	+156
9	-48	+4
10	-34	+3564

Table 5: Nasal cycle patency status as determined by spirometry before and after NO measurement.

Nasal cycle status		
Volunteer	Patent side before measurement	Patent side after measurement
1	Right	Right
2	Right	Right
3	Left	Left
4	Right	Right
5	Left	Left
6	Right	Right
7	Left	Left
8	Right	Right
9	Right	Left
10	Left	Left

Figures

Figure 1

Participant measuring peak exhaled nasal airflows using two spirometers with nasal adaptors.

Figure 1

Participant measuring peak exhaled nasal airflows using two spirometers with nasal adaptors.

Figure 2

Comparison of mean inhaled nasopharyngeal NO concentrations for all three breathing states tested. = bilateral nostril breathing, = congested-side unilateral nostril breathing, = patent-side unilateral nostril breathing.

Figure 2

Comparison of mean inhaled nasopharyngeal NO concentrations for all three breathing states tested. = bilateral nostril breathing, = congested-side unilateral nostril breathing, = patent-side unilateral nostril breathing.