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Documentation of the nasal nitric oxide response to humming: methods evaluation

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Abstract

Rationale Nitric oxide (NO) is present at higher concentrations in the nasal cavity than in the lower airway, and at even higher concentrations within the paranasal sinuses proper. When the paranasal sinus ostia are patent, acoustic activity produced by vocalization with closed lips (humming) promotes mixing of sinus with nasal gases, producing a further increase in nasal NO. We wished to evaluate procedures for the documentation of the nasal NO response to humming.

Materials and methods We compared two ATS-recommended sampling methods: 1) active exhalation of lower airway gas (parallel technique) and 2) passive aspiration of nasal gas with closed velopharynx (series technique). Variables controlled for included sampling rate, external resistance (parallel method), humming frequency, humming duration, and intertrial interval. Prior to upper airway sampling, exhaled lower airway NO was determined utilizing ATS-standardized technique.

Results Ten volunteers (seven males and three females, aged 21–58) with no history of respiratory allergies or sino-nasal disease were studied in a single session each. The parallel technique documented an increase in nasal NO during the humming manoeuvre in all subjects (mean ratio of humming-to-quiet NO, 4·2), whereas the series technique did so in eight of 10 subjects (mean ratio 2·1). Correcting for admixture from the lower airway, the ratio of humming-to-quiet NO was greater with the parallel than series sampling technique ($P < 0{\cdot}05$).

Conclusions Documentation of the response of nasal NO to humming in subjects without sino-nasal disease was consistently achievable by parallel sampling using commercially available equipment. Specific operational procedures are proposed.

Keywords Acoustic mixing, nasal cavity, nitric oxide, paranasal sinuses, sinusitis.
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Introduction

Nitric oxide (NO) is a naturally occurring biogenic gas present at higher concentrations in the upper than in the lower airway in humans. Although its physiological functions are incompletely understood, in the upper airway it is believed to have local antimicrobial activity, as well as influencing mucociliary function [1–3]. An apparent reservoir of NO in the upper airway is in the paranasal sinuses, where concentrations can reach thousands of parts-per-billion (several orders of magnitude higher than those normally found in the lower airway) [4,5]. The relative contribution to measured nasal NO by gas originating in the paranasal sinuses versus gas originating directly from the nasal mucosa is a subject of active investigation [6,7].

Relatively recently, investigators noted that the acoustic activity produced by vocalization with closed lips (humming) promotes mixing of sinus with nasal gases, thus producing a large 'spike' in NO concentration during real-time sampling [8,9]. This spike is dependent upon the patency of the paranasal sinus ostia, without which there is no communication between the sinuses and nasal airway [10,11]. Thus, documentation of the nasal NO response to humming holds promise for use as a surrogate index

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Table 1 Published study techniques for documenting the nasal NO response to humming

Reference	Sampling technique	Flow (L m^{-1})	Pressure	Time (s)	Compensation
8,9	Parallel	12	NS	5–10	nasal NO_{corr} = nasal NO_{uncorr} –FE_{NO}
13	Parallel	NS	NS	NS	NS
15	Parallel	Variable	NS	10–20	None
16	Series	0·7–1·2	NA	10	None
17	Series	0·7	NA	10	None

Nasal NO_{corr} = nasal NO corrected; nasal NO_{uncorr} = nasal NO (measured).
FE_{NO} = exhaled (lower airway) NO.
NA = not applicable.
NS = not specified.

of paranasal sinus ostial patency, impairment of which is thought to be an important factor in the pathogenesis of sinusitis.

The American Thoracic Society (ATS) has issued two consensus statements (1999, 2005) and one workshop (2006) on airway NO measurement, each mentioning nasal NO sampling [12–14]. The initial document advocated nasal NO sampling by passive aspiration across both nasal cavities in series (series sampling), with velopalatine closure to exclude lower airway gases (which contain NO at concentrations exceeding ambient levels). Acceptable sampling rates ranged from 3 to 5 L min^{-1} [12].

Recognizing the impossibility of maintaining velopalatine closure during the humming manoeuvre, the ATS in 2005 recommended 'parallel' sampling (active exhalation through both hemi-nasal cavities simultaneously) when the humming manoeuvre was to be employed [13]. However, the parameters to be employed (flow rate, external flow resistance, frequency and duration of humming, correction for the contribution of exhaled lower airway NO) were not specified, and actual techniques vary greatly among published studies [9,15–18]; Table 1.

Our objective in this study was to systematize the various operational variables involved in documenting the humming-induced spike in nasal NO in order to optimize the reproducibility of this diagnostic manoeuvre. The null hypothesis was that series and parallel sampling techniques would be equally efficacious in documenting the nasal NO response to humming in normal subjects without a prior history of allergic/sino-nasal disease.

Materials and methods

Subject recruitment

Volunteer subjects were recruited from a university complex. Inclusion criteria included age 18–60 years and good general health. Exclusion criteria included active smoking, self-reported allergic rhinitis and/or sinusitis (lifetime history), and cardiopulmonary diseases such as asthma or COPD (chronic obstructive pulmonary disease). Ten volunteers (seven males and three females, aged 21–58 years) participated after reviewing and signing an informed consent document approved by the University of Washington.

Equipment

Sampling was performed using a commercial chemiluminescent NO analyser (Sievers Model 280i, GE Analytical Instruments, Boulder, CO, USA), along with either: 1) a Teflon sampling wand connected to a vacuum rotameter (Dwyer Instruments, Michigan City, IN, USA; 'series method') or 2) a commercially available flow-sensing apparatus designed for on-line NO sampling (Model 4000, TSI, Shoreview, MN, USA; 'parallel method'). Alternate restrictors for the flow sensor were obtained from the same manufacturer (TSI). Sampling occurred with continuous on-screen feedback (REB software Version 3·21, GE Analytical Instruments, Boulder, CO, USA) on a laptop computer. Sampling parameters utilized to determine (lower airway) fractional exhaled NO (FE_{NO}), nasal NO by the parallel technique, and nasal NO by the series technique are summarized in Table 2.

Study procedures

Two-point calibration (0 and 45 ppm) was performed on the NO analyser on each testing day. Subjects were asked to avoid eating for one hour prior to testing, caffeine-containing beverages for two hours, and strenuous exercise for two hours. Note was made of the ambient (laboratory) NO concentration (A_{NO}) on each testing day. Subjects were first coached on maintaining the target humming frequency using an audio oscillator and microphone connected to the X and Y axes of an oscilloscope. Subjects practiced up to three times synchronizing their humming with a 128 Hz sign wave. They were then instructed to avoid unnecessary verbalization and were given instructions regarding study procedures. Baseline (lower airway) FE_{NO} was then sampled by having subjects blow through a mouthpiece and bacteriological filter at 3 L m^{-1} per ATS standards [12,13].

Table 2 Sampling parameters employed during lower airway and nasal NO sampling

Technique	(Lower airway) FE_{NO}	Nasal NO (parallel)	Nasal NO (series)
Interface	Mouthpiece	Mask	Probe
Flow rate	3 L min^{-1}	5 L min^{-1}	3 L min^{-1}
External resistance	33 340 Pa L^{-1} s^{-1}	9450 Pa L^{-1} s^{-1}	N/A
Frequency of humming:	N/A	128 Hz	128 Hz
Duration of humming:	N/A	≥ 10 s	≥ 10 s
Inter-trial interval	N/A	4 min	4 min

Parallel sampling

The restrictor on the sampling wand was changed from the default for FE_{NO} sampling (#2; 33 340 Pa L^{-1} s^{-1} at 3 L min^{-1}) to the next most patent (#3; 9450 Pa L^{-1} s^{-1} at 5 L min^{-1}), and a face mask (King System, Noblesville, IN, USA) was substituted for the mouthpiece. Subjects then inhaled orally through the mask/wand (which is equipped with a NO-scrubbing filter on its intake), and exhaled nasally through the mask with on-screen visual feedback to maintain a flow rate of approximately 5 L m^{-1}. Both flow and real-time nasal NO concentrations were recorded during this manoeuvre, which was repeated three times with quiet exhalation. Next, subjects inhaled orally through the mask and wand and exhaled until the investigator signalled that a steady NO plateau had been reached, at which time subjects began humming at target frequency, maintaining the same flow rate. Humming duration was at least 10 s, and a 4 min recovery time (with the subject abstaining from verbalization) was allowed between each of the 3 humming trials.

Series sampling

This technique utilizes a custom-fabricated Teflon sampling probe connected to a metered vacuum line operating at 3 L min^{-1}, with the NO analyser drawing gas from a T-connector. Subjects were instructed to take a deep breath in through the mouth, achieve velopalatine closure by making the 'k' sound, then to place the rounded end of the probe against the outside of one nostril and hold their breath for 15 s. During sampling air was drawn through the two hemi-nasal cavities in series, passing across the nasopharynx. Three such manoeuvres were repeated quietly. Next, subjects repeated the procedure, but were instructed to hum for at least 10 s once the investigator signalled that a steady nasal NO plateau had been reached. This manoeuvre was repeated twice more, with a 4 min interval between trials.

Finally, subjects were asked to simulate their series humming manoeuvre through a nosepiece which was connected to a pneumotachometer (Hans Rudolph; Kansas City, MO, USA) differential pressure transducer and signal conditioner (Validyne Engineering, Northridge, CA, USA); the opposite nostril was occluded during this manoeuvre. An estimate of steady-state flow during the series humming manoeuvre was made using a calibration curve generated using a precision flow meter (Cole-Parmer, Vernon Hills, IL, USA).

Data analysis

For each NO tracing, area-under-the-curve (AUC) and average NO concentration were estimated for a 10 s period using Image J software version 1·36b (NIH, Bethesda, MD, USA). Data were analysed using JMP Version 6 software (SAS Institute, Carey, NC, USA).

Raw data were treated in the following manner to obtain corrected humming-to-quiet (H/Q) nasal NO ratios:

For parallel sampling, lower airway FE_{NO} concentrations were deducted from both quiet and humming nasal NO:

$$\text{Parallel H/Q corrected} = [(PH - FE_{NO})/(PQ - FE_{NO})]^{\star}$$

For series sampling, A_{NO} (background NO concentration) was deducted from quiet nasal NO, whereas lower airway (FE_{NO}) concentrations were deducted from humming nasal NO:

$$\text{Series H/Q corrected} = [(SH - FE_{NO})/(SQ - A_{NO})]^{\star}$$

*Where:
A_{NO} = Ambient (laboratory) NO
FE_{NO} = Fractional exhaled (lower airway) NO
PH = Parallel humming nasal NO
PQ = Parallel quiet nasal NO
SH = Series humming nasal NO
SQ = Series quiet nasal NO

Statistical analysis

Data were checked for normality, and both the arithmetic and geometric mean values reported, and data log-transformed, when significant skew was detected. Group comparisons (parallel versus series sampling) were performed using either analysis of variance or non-parametric tests (Wilcoxon rank-sum or Wilcoxon signed-rank), as appropriate.

Results

Crude data for the experiment appear in Table 3. The mean background concentration of NO in the laboratory at the

Table 3 Raw data from experiment

Subject #	Age	Gender	SQ (ppb)	SQ_COV	SH (ppb)	SH_COV	SH/SQ	PQ (ppb)	PQ_COV	PH (ppb)	PH_COV	PH/PQ	FE_{NO} (ppb)	$\dot{V}$hum (L min^{-1})	A_{NO} (ppb)
1	57	M	100	3%	356	11%	3·57	67	10%	205	4%	3·07	31·3	5·6	3·0
2	58	F	86	6%	437	15%	5·09	62	2%	502	20%	8·06	15·4	3·8	10·5
3	52	M	223	3%	395	9%	1·77	52	14%	208	12%	4·02	28·3	6·2	4·1
4	35	M	283	5%	514	22%	1·82	211	5%	347	15%	1·65	48·8	4·2	18·0
5	21	F	132	4%	300	12%	2·27	87	13%	182	8%	2·09	14·0	6·2	20·0
6	38	F	115	0%	76	5%	0·67	71	9%	130	5%	1·8	28·3	7·1	24·0
7	40	M	96	3%	203	36%	2·13	53	9%	125	10%	2·34	22·5	8·0	12·0
8	49	M	122	18%	62	5%	0·51	29	16%	436	14%	15·2	27·5	4·5	4·2
9	41	M	107·1	1%	136	12%	1·27	62	4%	95	1%	1·53	36·4	5·3	2·5
10	57	M	140	5%	241	13%	1·72	109	2%	242	9%	2·22	62·9	4·5	7·0

Key to abbreviations:
A_{NO}, Ambient NO. [background lab NO concentration]
COV, Coefficient of variation. [SD/mean]
FE_{NO}, Exhaled (lower airway) NO. [Per ATS sampling standard]
PH, Parallel humming. [Nasal NO during...]
PQ, Parallel quiet.
SH, Series humming.
SQ, Series quiet.
$\dot{V}$hum, Airflow during humming. [pneumotachometer measurement]

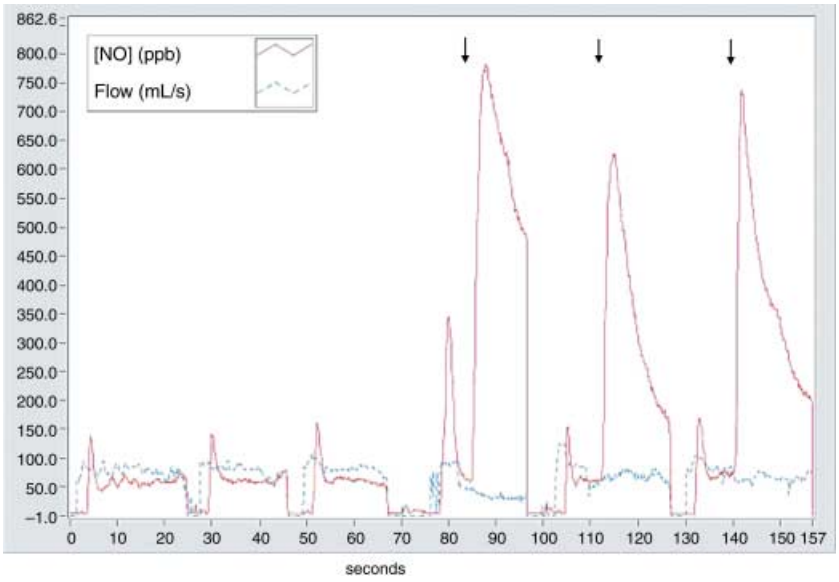

Figure 1 Humming-induced 'spike' in nasal NO: Parallel sampling method. Arrows indicate beginning of humming manoeuvre. Smaller spikes reflect positive pressure transient at beginning of each sampling run. Inter-trial interval for humming manoeuvres (4 min) is obscured by intermittent data logging.

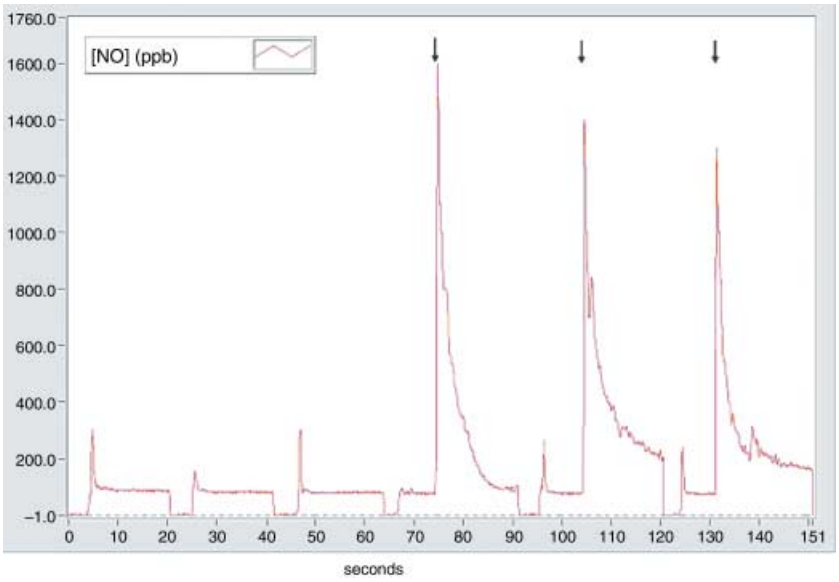

Figure 2 Humming-induced 'spike' in nasal NO: Series sampling method. Arrows indicate beginning of humming manoeuvre. Smaller spikes reflect negative pressure transient at beginning of each sampling run. Inter-trial interval for humming manoeuvres (4 min) is obscured by intermittent data logging.

time of sampling was 10·5 (± 7·8 SD) ppb, and the mean exhaled lower airway concentration across subjects was 31·5 (± 14·9 SD) ppb. Nasal NO measured under baseline (quiet) conditions was log-normally distributed, and significantly lower using the parallel technique – mean 80 (± 51 SD) ppb/70 ppb geometric mean, than the series technique, mean 140 (± 63 SD) ppb/130 ppb geometric mean ($P < 0{\cdot}01$ by Wilcoxon rank sums). For quiet sampling, the mean coefficient of variation across subjects (i.e. mean of individual SD/mean) was 8% for the parallel and 5% for the series technique.

During humming, all 10 subjects showed an increase in uncorrected nasal NO using parallel sampling, whereas only eight of 10 showed an increase using series sampling. For series sampling, all estimates of Vhum (airflow during humming) exceeded the series sampling rate (3 L min^{-1}) justifying the use of exhaled FE_{NO}, rather than ambient NO, as the compensatory deduction during series humming manoeuvres. Representative tracings for the parallel and series techniques appear in Figs 1 and 2, respectively. In general, the shape of the humming-induced 'spike' was broader using the parallel technique.

For each technique, the ratio of humming-to-quiet (H/Q) nasal NO was computed on both a crude and corrected basis (see above). The distributions of both of these metrics differed significantly from normal, resulting in their log transformations. Scattergrams comparing individual values of the two log-transformed metrics appear in Fig. 3 (uncorrected H/Q) and Fig. 4 (corrected H/Q). With the exception of two cases in which nasal NO actually decreased during

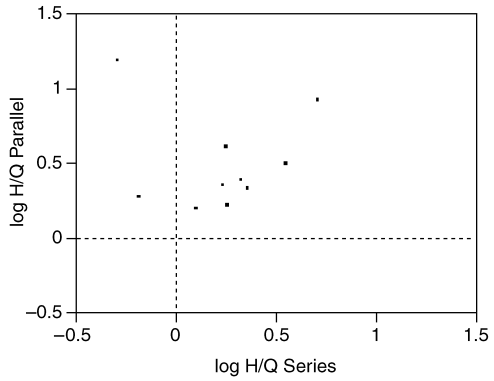

Figure 3 Scattergram of $\log_{10}$ (humming/quiet nasal NO uncorrected): parallel versus series sampling methods. Note two cases in which the series method yielded (humming/quiet nasal NO) < 1 (log value < 0).

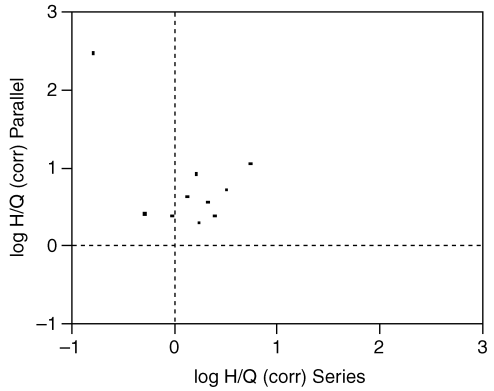

Figure 4 Scattergram of $\log_{10}$ (humming/quiet nasal NO corrected): parallel versus series sampling methods. Note three cases in which the series method yielded (humming/quiet nasal NO corrected) < 1 (log value < 0).

humming using the series technique (three cases when considering corrected nasal NO levels), the remaining values of the two metrics varied on a roughly proportional basis.

The mean ratio of humming-to-quiet nasal NO on an uncorrected basis was 4·2 (geometric mean 3·1) for parallel sampling and 2·1 (geometric mean, 1·7) for series ($P = 0{\cdot}13$ by Wilcoxon rank-sums). The corresponding corrected values were mean 31·1 (geometric mean, 5·6) for parallel sampling and 2·0 (geometric mean, 1·4) for series. The distribution of these latter values differed significantly ($P < 0{\cdot}05$ by Wilcoxon rank-sums). Additionally, we performed a paired analysis, comparing parallel versus series H/Q corrected by subject [Fig. 5]. In all but two of the cases, parallel H/Q corrected was greater than series H/Q corrected ($P < 0{\cdot}01$ by Wilcoxon signed-rank). In sum, the magnitude of the humming-induced 'spike' in nasal NO was significantly greater, on average, using the parallel rather than the series sampling technique once correction was made for admixture of exhaled lower airway and/or ambient gases.

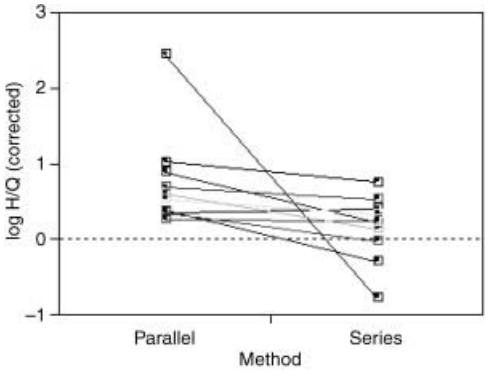

Figure 5 Paired analysis: Comparison of sampling methods. The ratio of (humming/quiet nasal NO corrected) was significantly greater with the parallel than the series method ($P < 0{\cdot}01$ by Wilcoxon signed-rank).

Discussion

Previous ATS recommendations have been to use the series sampling method for static nasal NO measurements, and the parallel method to document the nasal NO response to humming [17,18]. However, recommended sampling parameters have been more completely specified for the former than for the latter case, and adherence to recommendations has been inconsistent in the published literature. In our study, both parallel and series techniques demonstrated substantial humming-induced 'spikes' in nasal NO in the majority of normal subjects, although in two cases the results were discordant, the parallel method only showing this finding.

To our knowledge this is the first study to compare the performance of these two sampling techniques, particularly by performing tandem measures in the same subjects. A strength of this study, in addition to its overall design, was its relatively strict control over several hitherto inconsistently addressed sampling parameters, including humming frequency and duration, flow resistance (in the case of parallel sampling), intertrial interval, and flow rates. For example, while Maniscalco *et al*. (2003) explored the effect of humming frequency on NO flux from the sinuses (finding higher flux at 130 Hz than at either 150 or 450 Hz), they stopped short of advocating a specific frequency standard. In our case we chose 128 Hz because it was a fundamental frequency that was easily reproduced in both men and women of varying ages, and a 128 Hz calibration standard (tuning fork) is widely available as a medical examination tool.

Limitations of this study, on the other hand, include the relatively small sample size, the fact that we classified subjects' respiratory health based upon self-report, and by the consistent order of testing (parallel before series sampling). Although this last factor brings up the possibility of a testing order effect, we did not see a systematic diminution in NO AUC with humming across trials (data not shown), arguing that the 4 min intertrial interval was sufficient to permit relatively complete replenishment of sinus NO concentrations.

Finally, our sampling rate for lower airway gas (3 L min^{-1}) was matched to the series sampling rate rather than the parallel sampling rate. This decision in part reflected the fact that the REB software's variability sensing routine was contingent upon sampling at 3 L min^{-1}, which it considers 'ATS standard.' Ultimately, the flow rate for documenting nasal NO and exhaled FE_{NO} should be matched, more likely at 5 L min^{-1} than 3 L min^{-1} given the Vhum values obtained from our subjects (mean Vhum, 5·5 L min^{-1}). Of note, the parallel sampling rate cited by the Lundberg laboratory (12 L min^{-1} [8,9]); would not have been sustainable by many of our subjects for a 10 second period of humming, particularly since humming was not initiated until quiet nasal NO levels had stabilized at the beginning of each expiratory run.

Uncorrected, the ratio of humming-to-quiet nasal NO, measured as an average over 10 s of humming, tended to be greater using the parallel than series methods (geometric mean, 3·1 vs. 1·7; $P = 0{\cdot}13$ by Wilcoxon rank sums). Correcting for admixture of ambient air and/or exhaled lower airway gas, the ratio of humming-to-quiet nasal NO was significantly greater using the parallel method (geometric mean, 5·6 vs. 1·4; $P < 0{\cdot}05$). Perhaps more important than any statistical comparison, however, was the consistency of the 'spike' finding with the parallel, but not series, method. We thus find ourselves in agreement with the ATS recommendation based upon our structured comparison of techniques.

The anomalous finding of a reduction of nasal NO with humming, as documented in two cases using the series sampling method, begs explanation. Whereas the total flow rate with the series and parallel methods differed substantially (3 L min^{-1} vs. 5 L min^{-1}), the average flow rate through each hemi-nasal cavity was more closely matched (3 L min^{-1} vs. 2·5 L min^{-1}, assuming equal partitioning of flow with the parallel method). At baseline (i.e. with quiet exhalation), the series method yielded higher nasal NO concentrations than did the parallel method. This argues that the serial augmentation of NO in sampled gas across the two hemi-nasal cavities was important in achieving this result. Loss of sampling from the opposite hemi-nasal cavity during humming may have offset any augmented contribution from the sinuses in these two cases, despite the fact that exhaled lower airway NO concentrations exceeded ambient concentrations.

Paranasal sinus ostial obstruction, whether from infection, allergy, or inhaled irritants, is thought to be a part of the pathophyisiological chain resulting in the development of sinusitis, an inflammatory upper airway condition with serious associated morbidity and costs [18–20]. Standardization of the method for documenting the nasal NO response to humming would help further the use of this non-invasive technique as a surrogate for paranasal sinus ostial patency in both diagnostic and pathophysiological studies of sinusitis.

References

1 Lundberg JO. Airborne nitric oxide: inflammatory marker and aerocrine messenger in man. *Acta Physiol Scand Suppl* 1996;**633**:1–27.

2 Alberty J, August C, Stoll W, Rudack C. The effect of endogenous nitric oxide on cholinergic ciliary stimulation of human nasal mucosa. *Laryngoscope* 2004;**114**:1642–7.

3 Runer T, Lindberg S. Ciliostimulatory effects mediated by nitric oxide. *Acta Otolaryngol* 1999;**119**:821–5.

4 Lundberg JO, Farkas-Szallasi T, Weitzberg E, Rinder J, Lidholm J, Anggaard A *et al.* High nitric oxide production in human paranasal sinuses. *Nat Med* 1995;**1**:370–3.

5 Andersson JA, Cervin A, Lindberg S, Uddman R, Cardell LO. The paranasal sinuses as reservoirs for nitric oxide. *Acta Otolaryngol* 2002;**122**:861–5.

6 Lundberg JO, Rinder J, Weitzberg E, Lundberg JM, Alving K. Nasally exhaled nitric oxide in humans originates mainly in the paranasal sinuses. *Acta Physiol Scand* 1994;**152**:431–2.

7 Haight JS, Djupesland PG, Qjan W, Chatkin JM, Furlott H, Irish J *et al.* Does nasal nitric oxide come from the sinuses? *J Otolaryngol* 1999;**28**:197–204.

8 Weitzberg E, Lundberg JO. Humming greatly increases nasal nitric oxide. *Am J Respir Crit Care Med* 2002;**166**:144–5.

9 Maniscalco M, Weitzberg E, Sundberg J, Sofia M, Lundberg JO. Assessment of nasal and sinus nitric oxide output using single-breath humming exhalations. *Eur Respir J* 2003;**22**:323–9.

10 Lundberg JO, Maniscalco M, Sofia M, Lundblad L, Weitzberg E. Humming, nitric oxide, and paranasal sinus obstruction. *JAMA* 2003;**289**:302–3.

11 Maniscalco M, Sofia M, Weitzberg E, De Laurentiis G, Stanziola A, Rossillo V *et al.* Humming-induced release of nasal nitric oxide for assessment of sinus obstruction in allergic rhinitis: pilot study. *Eur J Clin Invest* 2004;**34**:555–60.

12 ATS (American Thoracic Society). Recommendations for standardized procedures for the on-line and off-line measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children-1999. *Am J Respir Crit Care Med* 1999;**160**:2104–17.

13 ATS (American Thoracic Society). ATS/ERS Recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005;**171**:912–30.

14 Silkoff PE, Erzurum SC, Lundberg JO, George SC, Marczin N, Hunt JF *et al.* ATS workshop proceedings: exhaled nitric oxide and nitric oxide oxidative metabolism in exhaled breath condensate. *Proc Am Thorac Soc* 2006;**3**:131–45.

15 Menzel L, Hess A, Bloch W, Michel O, Schuster KD, Gabler R *et al.* Temporal nitric oxide dynamics in the paranasal sinuses during humming. *J Appl Physiol* 2005;**98**:2064–71.

16 Struben VM, Wieringa MH, Mantingh CJ, Bruinsma SM, de Jongste JC, Feenstra L. Silent and humming nasal NO measurements in adults aged 18–70 years. *Eur J Clin Invest* 2005;**35**:653–7.

17 Struben VM, Sewbalak WV, Wieringa MH, Mantingh CJ, van den Toorn LM, Bakker M *et al.* Nasal nitric oxide in cystic fibrosis with and without humming. *Eur J Clin Invest* 2007;**37**:140–4.

18 Bhattacharyya N. The economic burden and symptom manifestations of chronic rhinosinusitis. *Am J Rhinol* 2003;**17**:27–32.

19 Murphy MP, Fishman P, Short SO, Sullivan SD, Yueh B, Weymuller EA Jr. Health care utilization and cost among adults with chronic rhinosinusitis enrolled in a health maintenance organization. *Otolaryngol Head Neck Surg* 2002;**127**:367–76.

20 Ray NF, Baraniuk JN, Thamer M, Rinehart CS, Gergen PJ, Kaliner M *et al.* Healthcare expenditures for sinusitis in 1996: contributions of asthma, rhinitis and other airway disorders. *J Allergy Clin Immunol* 1999;**103** (3 Part 1):408–14.