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Aspiration flow optimized for nasal nitric oxide measurement*

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SUMMARY

The aim of the present study was to evaluate some of the factors which may influence the reliability of nasal NO measurements, and to optimize methods suitable for children and adults. Nasal nitric oxide (NO) output was determined by chemiluminescent analysis of aspirated samples in 16 adults and 6 children. With the velopharyngeal aperture closed, stable NO levels were obtained at flows ranging from 0.9 to 6.2 L/min. NO output averaged 401.0±145.4 nL/min./M^2 in 6 children, 338.2±92.3 in 7 adult females and 268.6±70.2 in 9 adult males. Nasal NO output was independent of flow provided a stable plateau of NO value was reached. In this study, the optimal range of flows was 3.2-5.2 L/min. in adults and 2.2-3.2 L/min. in children. This enables selection of the most favorable flow to be chosen for individual subjects and situations.

Key Words: Nasal cavity, nitric oxide, nasal volume, acoustic rhinometry, children

INTRODUCTION

The ability of mammalian cells to synthesize an endothelially-derived relaxant factor (EDRF) was first demonstrated by Furchgott and Zawadski in 1980 (Furchgott and Zawadski, 1980). Subsequent investigations have shown that the actions of EDRF and nitric oxide (NO) were substantially similar (Ignarro et al. 1987; Palmer et al. 1987). NO is synthesized from the terminal guanidine nitrogen of the semi-essential amino acid L-arginine, which is converted to L-citrulline in a stereo-specific reaction catalyzed by a family of nitric oxide synthases (NOS) (Singh and Evans, 1997). It has been shown that NO is an important inter- and intracellular mediator, governing several physiological functions in animals and humans, ranging from control of smooth muscle tone in cardiovascular, gastrointestinal, respiratory and genitourinary systems, to neurotransmission and a role in immune function and inflammation (Singh and Evans, 1997).

NO was first detected in the nasal airways by Alving (Alving et al. 1993). Later studies have shown that exhaled NO originates mainly from upper airways with only a minor contribution from the lower airways and lungs in healthy subjects(Lundberg, 1996a). The high local NO concentration in the nasal airways and the paranasal sinuses may help to protect against airborne infectious agents. Thus, airway NO may represent the very first line of defense in the airways, possibly acting on pathogens even before they reach the mucosa (Lundberg, 1996a).

It has been suggested that measurement of exhaled NO may be clinically useful in non-invasive diagnosis and monitoring of

Table 1. Nasal NO output reported by several studies.

Author, year	Method	Flow rate (L/min)	NO output (nL/min)
Lundberg, 1994	NA + VO	0.8	176.8
Schudin, 1995	NA + VO	0.57	224.6
Kimberly, 1996	NI + VC	4.0-6.0	468
Lundberg, 1996	NA + VC	0.7	240.8
Imada, 1996	NA + VC	0.5-2.0	323
Rinder, 1996	NA + VO	0.7	234.5
Kharitonov, 1997	NA + VC	0.25	249
Ferguson, 1997	NA + VC	0.25	255
Baraldi, 1997	NA + VO	0.7	171.5
DuBois, 1998	NA + VC	0.082-0.347	352
Present study			
male adults	NA + VC	2.2-6.2	510
female adults	NA + VC	2.2-6.2	546.2
children	NA + VC	1.2-3.2	372.1

NA: nasal aspiration, NI: nasal insufflation VC: vellum closed, VO: vellum opened.

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inflammatory airway diseases (Singh and Evans, 1997; Lundberg, 1996a), and several methods have been introduced for nasal NO assessment (Kharitonov et al. 1997; Silkoff et al. 1998). However, a wide variation of NO values reported has not provided a firm basis for the clinical use of this form of measurement (Table 1) (Baraldi et al. 1997; DuBois et al. 1998; Ferguson and Eccles, 1997; Imada et al. 1996; Kharitonov et al. 1997; Kimberly et al. 1996; Lundberg et al. 1996b; Lundberg et al. 1994; Rinder et al. 1996; Schedin et al. 1995).

The aim of the present study was to evaluate some of the factors which may influence the reliability of nasal NO measurements, and to optimize methods suitable for children and adults.

MATERIALS AND METHODS

Sixteen healthy adults ranging in age from 19 to 53 years (mean ±SD 35.1±11.1, 9 males) and 6 children ranging from 5 to 10 years (mean ±SD 8±2.2, 3 males) were recruited for this study. Subjects had no history of a recent upper airway infection, allergy, structural nasal pathology or were using corticosteroids. All subjects were not smokers. All subjects provided informed consent approved by Human Ethics Committee of the University of Toronto.

Measurement of NO and sampling techniques

NO was measured by means of a rapid-response chemiluminescent NO analyzer (Sievers 280, Boulder, CO.). The sampling flow of the vacuum pump was 0.2 L/min. A daily two-point calibration was performed, first with 100% nitrogen to zero, then with an analyzed standard gas (NO 1.6 ppm) for the span. Ambient air NO was recorded before and after each subject was studied. Zero and span gases were checked periodically. The NO analyzer signal output was fed to a computer data acquisition program (DasyLab for Windows, DasyTec Corp. Amherst, NH, USA) with a real-time chart recorder like display of NO versus time written directly to the computer's hard disk as a data file. NO concentration were measured using a data analysis program, written in-house (Microsoft Visual Basic). The NO concentration used to calculate the nasal NO output was obtained by subtraction of NO ambient from measured NO levels. This was justified by a series performed in 3 subjects evaluating the effect of ambient NO levels (ranging from 0 to 150 ppb) on nasal NO concentration. Nasal NO output (nL/min./M^2) was calculated from the NO concentration (ppb), flow rate (L/min.) and corrected for body surface area (M^2) (Diem and Lentner, 1970). The statistic analyses was performed by the GraphPad PRISM software, using ANOVA and Student's t test (paired and unpaired). Statistical significant was considered a $p < 0.05$.

1. Nasal NO measurement in adults (n=16)

A Teflon nozzle was positioned gently inside a nasal vestibule to provide an airtight seal while the opposite nostril remained open. Air was aspirated from the nose at flows of 0.9, 2.2, 3.2, 4.2, 5.2, and 6.2 L/min. In order to ensure closure of the velopharyngeal orifice during measurement, subjects were instructed to expire orally through an airflow resistor after taking a deep inspiration. Thus, with the velopharyngeal aperture closed, room air was aspirated at adjusted flow through both nasal cavities and the nasopharynx in series. Meanwhile, NO sampling took place through a side-arm tube connected to the pump. Steady NO plateau, defined as a variation of NO concentration less than 5 ppb over a period at least 10 seconds (sec), was recorded. The means of 3 independent plateaus were used for further analysis and the time required to reach plateau at each maneuver was recorded. NO concentration of room air was recorded before and after each measurement.

2. Nasal NO measurement in children (n=6)

A method similar to "nasal cavities in series" described above was employed in the children using lower flows (1.2, 2.2 and 3.2 L/min.). However, younger children were unable to hold their breath for a sufficient length of time to achieve a steady NO plateau. Consequently, NO had to be measured during mouth breathing with these children. A preliminary experiment was also performed with children to assure the closure of the velopharynx in which a transparent tube was inserted in the nostril opposite to the aspirated side. Mist was observed in the tube during unrestricted breathing but remained clear during mouth breathing with the velopharynx closed.

3. Acoustic rhinometry

Nasal cavity dimensions were measured by means of a continuous wide-band noise acoustic rhinometer (Rhin2100, Rhinmetrics A/S, Lynge, Denmark, Software version 1.29) immediately following NO measurement. Subjects sat with head in stable position and breath held during each acoustic test. Three independent traces with less than 5% variation of the minimum cross section area (MCA) were accepted. The volumes from the nostril to the transition to the epipharynx as well as the volume over a distance of 2-5 cm from the nostril along the acoustic pathway were averaged.

RESULTS

1. Nasal NO output in adults

None of the subjects achieved a stable plateau of nasal NO concentration within the maximum breath holding at a flow of 0.9L/min. So did one subject at a flow of 2.2L/min. In 4 subjects, a steady NO concentration was not achieved within 30 sec breath holding at this flow. At the other extreme, several subjects experienced difficulty in reaching a stable plateau at flows of 5.2 and 6.2L/min. due to flow-induced alar collapse. Introduction of an open nozzle to the opposite nostril may reduce the tendency for collapse permitting use of a higher flow. NO output for flows (2.2 to 6.2 L/min.) in which a stable plateau was achieved averaged (±SD) 300.7±87.4 nl/min/M^2 (male 268.6±70.2, female 338.2±92.3, p=0.5.) (Table 2, Figure 1). There were no significant differences in NO output over the effective range of aspiration flows (ANOVA, p= 0.49, n=16) (Figure 2).The time required to reach a plateau varied inversely with the increasing of aspiration flow rate. An average (±SD) of 17.9±16.6 sec to reach a stable NO plateau at a flow of 2.2L/min. and 8.0±6.3 sec at a flow of 4.2L/min (unpaired t-test, p=0.024) (Figure 3).

Table 2. Nasal NO output* at different flows in adults.

	Flow**							Body***
Subj.	2.2	3.2	4.2	5.2	6.2	Mean	SD	Surface
1	283.3	284.4	280.0	252.1		275.0	15.4	1.98
2	315.1	295.0	304.1			304.7	10.1	1.92
3	310.4	324.9	313.9	319.0	319.6	317.6	5.6	1.94
4		400.5	406.0	425.2	404.4	409.0	11.0	1.59
5	499.5	524.5	492.1	467.7	524.6	501.7	24.0	1.69
6	96.0	99.3	101.1	94.9	100.2	98.3	2.7	2.16
7	282.7	251.6	249.3	266.1	265.2	263.0	13.4	1.87
8	305.5	280.4	313.9	301.9	283.3	297.0	14.5	1.86
9	222.5	221.2	229.3	215.2	224.5	222.5	5.1	1.74
10	258.0	228.1	294.4	284.1	288.6	270.7	27.6	1.94
11	407.4	374.7	350.8	365.9	348.3	369.4	23.9	1.62
12	254.5	262.3	254.0	266.0		259.2	5.9	1.72
13	293.3	275.9	324.7	313.8	324.3	306.4	21.3	1.74
14	306.1	280.9	268.2	300.7	283.9	288.0	15.4	1.66
15	314.1	296.9	323.9	330.5	308.9	314.8	13.1	1.66
16	331.2	333.6	332.9			332.6	1.3	1.88
Mean	298.7	295.9	302.4	300.2	306.3	300.7	3.9	1.83
SD	86.6	91.1	83.3	89.3	100.8			0.16
CV %	29	31	28	30	45			

*: nl/min/M^2 ; **: L/min; ***: M^2 ; blank: not reach a steady plateau

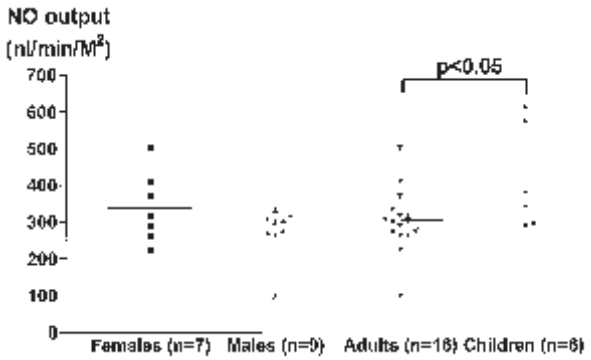

Figure 1. Mean nasal NO output in different groups. There was no significant difference between male and female group and it was significantly higher in children group compared to adults (P<0.05).

2. Nasal NO output in children

The flow of 1.2 L/min. was too low to achieve steady NO plateaus in 2 children. Mean NO output in this group was 401.0±145.4 nl/min/M^2 which was significantly higher than the NO output in adults (unpaired t-test, p<0.05) (Table 3) (Figure 1).

Table 3 Nasal NO output* in children.

	Flow**					Body***
Subj.	1.2	2.2	3.2	Mean	SD	Surface
1		373.7	385.6	379.7	8.4	0.8
2	567.7	570.1	578.1	572.0	5.4	0.9
3	278.3	311.3	292.8	294.1	16.5	0.9
4		641.1	578.1	609.6	44.6	0.9
5	293.9	276.5	296.5	289.0	10.9	1.1
6	356.0	341.0	328.5	341.8	13.8	0.9
Mean	374.0	418.9	409.9	401.0	23.8	0.92
SD	133.5	149.8	134.4	145.4		0.1
CV %	36	36	33	36		

*: nl/min/M^2; **: L/min; ***: M^2; blank: not reach a steady plateau

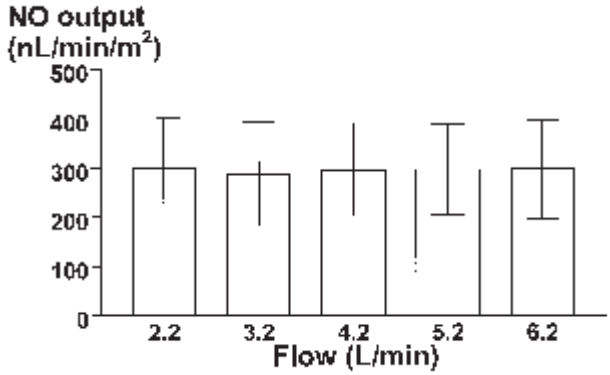

Figure 2. Mean(±SD) NO output at different flows. There was no significant difference in NO output at flows ranging from 2.2 to 6.2 L/min. (ANOVA, p=0.49, n=16)

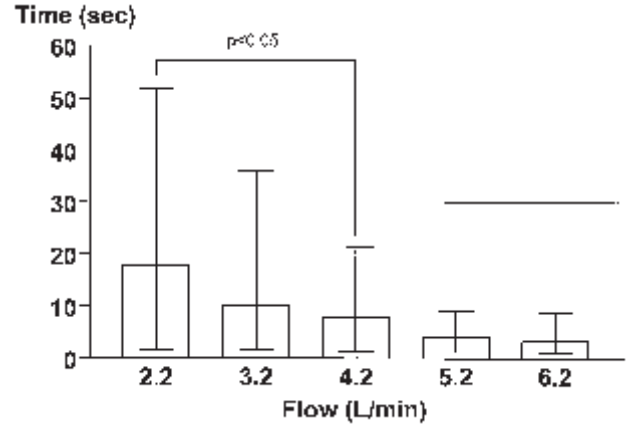

Figure 3. Time from the beginning of breath holding to reach a stable plateau. There was significant difference between 2L/min. and 4L/min. (p=0.024)

3. Acoustic rhinometry

The mean of depth of nasal airway and corresponding nasal volume in children and adults is shown in Table 4.

Table 4. Nasal volume and nasal airway depth in children and adults.

	nasal volume	nasal depth	body surface	nasal volume / body surface
children	8.02±1.28	6.11±0.43	0.92±0.1	8.72ml/M^2
adults	15.32±2.79	7.42±0.42	1.83±0.16	8.37ml/M^2

DISCUSSION

This study confirmed previous investigations showing that nasal NO concentration measured by sampling of air aspirated from the nasal cavities is influenced by the flow rate (DuBois et al. 1998; Imada et al. 1996; Lundberg, 1996a; Silkoff et al. 1998). It was also demonstrated that the time to reach a steady NO plateau should be always considered when aspirating from the nasal cavities. Previous studies report NO outputs varying from 171.5 to 468 nl/min (Baraldi et al. 1997; DuBois et al. 1998; Ferguson and Eccles, 1997; Imada et al. 1996; Kharitonov et al. 1997; Kimberly et al. 1996; Lundberg et al. 1996b; Lundberg et al. 1994; Rinder et al. 1996; Schedin et al. 1995). The large variation may be due to several factors. Methodological, physiologi-

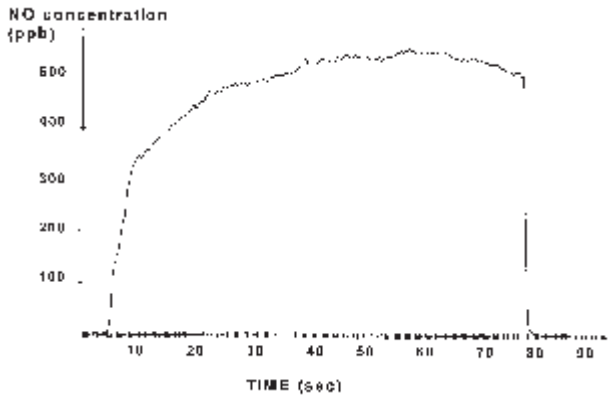

Figure 4. NO concentration kept rising and drooped after peak at a maximum breath holding, there is not a steady NO plateau. Flow: 0.9L/min.

cal and pathological factors might explain the differing results (Imada et al. 1996). The increased time needed to obtain a steady NO plateau when using low flows of the analyzer vacuum pump (0.25-0.8L/min.) represents a major problem (DuBois et al. 1998). When a flow of 0.9L/min. was used in this study, nasal NO concentration kept rising and dropped after the peak during the maximum breath holding (Figure 4). Even at a flow of 2.2 L/min., 4 subjects required more than 30 seconds, and one even more than 50 seconds, to reach a steady NO plateau. We found that it took an average (±SD) of 17.9±16.6 sec to reach a steady NO plateau at a flow of 2.2L/min. and 8.0±6.3 sec at a flow of 4.2L/min. A similar result was observed by DuBois (DuBois et al. 1998), who found that 2-5 min.was required to reach a steady state plateau at a sampling rate of 347ml/min. At the low flow rate of 8.2ml/min., a steady level was reached after 10-15 min., indicating that the time required to achieve a stable plateau was inversely related to flow (Figure 2). For clinical purposes, 30 sec was regarded as a practical period of breath holding for adults. A flow between 3.2 and 5.2 L/min was found to be suitable for achievement of a stable NO plateau within the period in this study. We found that within this range the NO output was independent from flow. This finding enables a choice to be made as to the most appropriate flow for each subject and situation. A low flow might be suitable in patients with a narrowed nasal airway, whereas a higher flow might be required to attain a stable plateau within a reasonable time after surgical intervention. In brief, we recommend the use of a flow sufficient to obtain a true steady plateau within less than 30 seconds.

In this study, we preferred nasal aspiration through a single nozzle to the insufflation technique (Chatkin et al. 1998; Kimberly et al. 1996), which required a nozzle in each nostril with correspondingly greater risk of leakage. Furthermore, aspiration aids sealing of the velopharyngeal aperture while insufflation has the opposite effect. The disadvantage of aspiration is flow-induced alar collapse at high flows, which may be counteracted by introducing a nozzle to the contralateral nostril. Isolation of the nasal airways during aspiration 'in series' requires closure of the velopharyngeal aperture. Two non-invasive methods for this purpose were introduced by previous studies: (a) oral Valsalva while breath holding and (b) voluntary closure of velopharyngeal aperture during mouth breathing (DuBois et al. 1998; Kimberly et al. 1996; Singh and Evans, 1997). The advantage of the latter was that subjects can tolerate a longer measurement period during procedure even up to 15 min. (DuBois et al. 1998; Kimberly et al. 1996). However, this technique requires training before the test, and some subjects are not able to master the maneuver. The present study employed an expiration against an oral resistor which assured velum closure with the added advantage of no training or discomfort.

Nasal NO measurement in children

Older children accepted adult measuring techniques without difficulty but in many cases younger children were unable to hold their breath for a period sufficiently long for a stable NO plateau to be attained. However, it was found that, in the 6 children tested, closure of the velopharyngeal aperture accompanied tidal mouth breathing quite spontaneously. This is helpful to establish a nasal NO measurement on younger children. Whether this method could be of use in infants remains to be determined.

The relationship between nasal volume and body surface was very similar for both adults and children (Table 4). The significantly greater NO output adjusted by body surface area in children ($p<0.05$) in our study could reflect either a greater production per square unit of nasal mucosal surface or a greater surface area relative to both nasal volume and body surface. The acoustically derived volume (15 ml) is very similar to the volume report in the literature (16-20 ml) estimating the total nasal mucosal surface to approximately 320 cm^2 (Paulsson et al. 1992). However, the relationship between volume and surface is not linear, particularly in the complex geometry of the nasal airway. Ventilation differences between adults and children prevent reliable conclusions regarding the relationship between NO output and age. Further studies are needed to clarify the previously suggested age-dependence of NO output (Lundberg et al. 1995). In order to ensure accurate and reproducible results of nasal NO measurement it is essential that aspiration flow be adequate to achieve a stable plateau of NO concentration within a reasonable breath holding period.

In this study, the optimal range of flows in adults lies between 3.2 and 5.2 L/min. and in children between 2.2 and 3.3 L/min. Since NO output is independent from flow, a flow most suitable for an individual patient can be chosen. The measurement flow rate can be customized to the patient's characteristic.

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