A human study model for nitric oxide research in sinonasal disease☆

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Sinus nitric oxide (NO) measurements present a novel and promising approach to help overcome difficulties and confounding variables associated with nasal NO measurements such as the nasal cycle, ostial patency, and individual contribution to total NO production of each sinus. Conflicting results reported on nasal NO measurements in various sinonasal diseases are presumed to originate from the variable diffusion of sinus NO into the nose where it is measured. This study presents a novel technique and research method for direct measurement of sinus NO. The authors’ original technique of individual, non-destructive catheterization of the sinuses through their natural ostia is developed and refined to allow accurate measurements of NO produced in the sinuses. Our study indicates that reproducible catheterization of the sinuses through their natural ostia can be performed in the clinical research setting under local and topical anesthesia. The model can be used to test the effects of various conditions on nasal and sinus NO production in a variety of disease models and the variables affecting sinonasal gas exchange can be differentially studied. Volunteer healthy adult human subjects without nasal allergies are used. An endoscopic nasal exam with topical anesthesia followed by in vitro allergy testing is performed to determine eligibility. Sinus computerized tomography (CT) scans are used to delineate anatomic features and to calculate paranasal sinus volumes. Continuous flow sinus air sampling and NO measurement with a chemiluminescence analyzer is obtained through polyethylene tube catheters (PEC) placed endoscopically into an aerated major paranasal sinus. Catheters are introduced through natural ostia under local and topical anesthesia. Nasal and differential sinus NO measurements are performed.

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1. Introduction

NO is a colorless, odorless gas that is formed in vivo from arginine by three isoforms of the enzyme nitric oxide synthase (NOS). NO acts as a final transmitter in many body systems. In the vascular system, brain and kidneys, NOS enzymes are permanently active and are called constitutive NO synthases (cNOS). In contrast, another isoform of NOS (iNOS) is inducible in response to inflammation and pathophysiological stress.

A large proportion of NO found in exhaled air originates from the upper airways, with only a minor contribution from...
the lower respiratory tract and the lungs [1–5]. There is continuous high production of NO in the human nasal passages and paranasal sinuses. Non-invasive measurements of nasal NO are easy to perform and have revealed altered levels in several respiratory disorders albeit with significant discrepancies between studies.

The biological significance of the sinus-derived NO is still uncertain. NO has been proposed as a bronchodilator [6], a vasodilator [7,8], a major neurotransmitter [9,10], and to have antimicrobial [11,12], antitumor [13,14] and mucociliary regulating activities [15–17] and also to act as an airborne messenger [18,19] with pro-inflammatory actions [20–22].

Exhaled NO measurement from the lower airway (FeNO) provides a simple, non-invasive means of measuring airway inflammation in asthma [23]. Levels of FeNO correlate with eosinophilic airway inflammation and raised levels predict steroid responsiveness [24–26]. FeNO measurements are as accurate as sputum eosinophilia in asthma diagnosis, with a sensitivity of 88% and specificity of 79%, but are simpler and accurate as sputum eosinophilia in asthma diagnosis, with a sensitivity of 88% and specificity of 79%, but are simpler and accurate as in the upper airway defense. Theoretically, nasal NO levels have been postulated to depend mainly on the amount of NO produced by the ciliated epithelium of the paranasal sinuses and the size of the paranasal sinus ostia [37]. The relative importance of the size of the individual sinus (volume–surface area) has not been addressed, since differential measurements of sinus NO have not been systematically studied or performed.

Adding to the complexity is the fact that swelling of the mucosa or accumulated secretions during inflammation may lead to decreased transport of NO from the paranasal sinuses to the nasal cavity where it is measured. In such cases, the net change in nasal NO will be variable and difficult to predict. The following studies reflect this complexity.

Nasal NO has been reported as decreased in acute rhinosinusitis [38], nasal polyposis [39], and cystic fibrosis [32,40], and as normal or increased in allergic rhinitis (AR) [41,35,34]. NO production in the nasal mucosa of patients with AR may be up-regulated. On the other hand, this increase could be counteracted by swelling of the mucosa and secretions resulting in impaired NO diffusion from the paranasal sinuses, where particularly high levels of NO have been found. Also, the high background levels of NO from constitutive sources in the nose may blunt smaller increases in mucosal NO output.

In chronic rhinosinusitis, Lindberg et al. [42] found lower nasal NO levels than in healthy controls; Arnal et al. [43] found no significant difference. Ragab et al. [44] found that nasal NO correlated well with computed tomography changes, and the percentage rise in nasal NO seen on medical and surgical treatment of chronic rhinosinusitis correlated with changes in symptom scores, saccharine clearance time, endoscopic changes, polyp grades and surgical scores. The authors suggested that nasal NO provides a valuable non-invasive objective measure of the response of chronic rhinosinusitis to therapy, but that topical nasal corticosteroids may be needed to reduce the contribution of nasal epithelial NO and allow that emanating from the sinuses to be measured. However this may not be sufficient since nasal steroids reduce nasal measurements of NO by 20% [45] and this effect may also be confounded by the vasoconstriction and improved ostial patency caused by the steroid. NO measurements obtained from individual sinuses addresses these variables.
Several techniques for measuring nasal NO have been used. The most common way to measure nasal NO is to sample nasal air directly from one nostril. Using the intrinsic sampling flow of a chemiluminescent analyzer or an external pump, air is aspirated from (or insufflated into) one nostril [46,47]. Guidelines for FeNO and nasal NO measurement have recently been produced [48]. Stark et al. [49] suggested that the measurement should be made at the same time of day. Struben et al. [50] found no significant diurnal variation. The discrepancy here may be due to the effects of the nasal cycle, introducing a high-level variable by nasal mucosal congestion preventing sinus NO from entering the nasal cavity.

The production of NO by different sinuses and the effect of intravenous arginine have been investigated in one volunteer [51] who was the researcher himself. This study was however not designed to explore parameters contributing to differential sinus and nasal NO production and mainly investigated the effects of gaseous carbon dioxide and intravenous arginine on NO measurements. The need for differential measurements of sinus NO production in defining NO exchange dynamics in the upper airway is unmet.

One of the more recent findings is the discovery that nasal NO increases dramatically (5- to 15-fold) during humming compared with silent nasal exhalation [52]. The current explanation for this phenomenon is that oscillating sound waves speed up the exchange of gases over the sinus ostium, resulting in a rapid washout of NO from the sinuses [53]. However, this theory appears weak with no explanation of how sound waves accomplish this. The theory also does not take into account that humming primarily is slow exhalation. NO levels during slow exhalation have not been studied. The humming-induced NO peak is transient and the levels decrease gradually during repeated humming maneuvers.

Nasal NO levels fully recover after a short period of silence, which allows sinus NO to accumulate again. In a model of the nose and sinus, it was found that ostium size was the main determinant of the humming-induced transient increase in NO. In addition, in patients with complete sinus ostial obstruction (bilateral nasal polyposis), the nasal NO increase during humming was abolished [54]. This suggests that the increase in nasal NO during humming correlates with ostial function. Ostial obstruction is central in the pathogenesis of sinusitis, and one goal in medical and surgical therapy of chronic rhinosinusitis is to improve sinus ventilation. Therefore humming has been suggested as a test of patency of the ostiomeatal complex [53]; it improves discrimination between healthy controls and children with cystic fibrosis better than static NO measurements [55]. Here again demonstrated is the importance of the dynamics of NO exchange between the sinuses and the nasal cavity, affecting the diagnostic accuracy of nasal NO measurements. Differential measurements are needed to assess the fractional contribution of sinus NO to nasal NO measurements, in variable conditions, including humming, which are in turn, likely to improve our understanding of NO dynamics in the upper airway.

A human study model for research on sinonasal disease using NO, a non-invasive objective marker, is the key to advancing our knowledge about the physiology, function and pathology of the sinuses. This study model can help describe and classify inflammation of the nose and sinuses in acute, chronic, bacterial, viral and other inflammatory diseases such as polyps. The study model can be used to design studies testing various hypotheses and will help to describe and validate objective diagnostic criteria for the differential diagnosis of different forms of rhinosinusitis and to describe key inflammatory pathways leading to the development of rhinosinusitis. New and advanced treatment methods may be developed based on our enhanced understanding of sinonasal pathophysiology. As a direct result of our observations during the study, a new aerodynamic theory of the nose is developed that explains sinonasal gas exchange dynamics, lends support to the role of NO as an aerocrine messenger, and has the potential to shape sinonasal surgical techniques (IFAR Int Forum on All Rhinol. Presented at COSM 2012 in San Diego and ERS-ISIAN 2012, Toulouse, France. Accepted for publication) [56,57].

2. Materials and methods

The study was supported by a grant from the Scott & White Research Grants Program at the Texas A&M Health Science Center as a Research Advancement Award for the primary investigator Anil A. Gungor MD (A Human Study Model for Research in Sinonasal Disease—Scott & White Institutional Review Board Registration #IRBO00000706 Project ID#: 90344) Protocol and consent forms were IRB approved and study was monitored by the IRB.

Subject inclusion criteria: Twelve adult volunteers; healthy subjects with no sinonasal disease, no anatomic sinonasal deformity, non-smoker; male or female; and age 18 to 65 years. Subject selection methods: Health history questionnaire, nasal symptom score questionnaire, physical and ENT exam, nasal endoscopic exam, and in vitro allergy testing by RAST method.

Research procedures: CT scan of the sinuses, nasal NO measurement, placement of sinus catheters under endoscopic guidance, with topical and local anesthesia, and sinus and nasal NO measurements. NO measurements are made with a chemiluminescence analyzer in real time.

Data collection: History and physical exam, sinonasal symptom questionnaire, allergy test results, and CT scans for delineating anatomy, planning for catheter placement, and calculation of individual sinus volumes.

NO measurements for nasal NO and sinus NO from individual sinuses during quiet breathing, breath-holding and humming maneuvers were performed. In addition to breath-holding, a Foley balloon catheter was used to seal the choana while sinus NO measurements were performed.

Nasal NO production was measured as ppb, with 3 l/min airflow through nasal passages in parallel and 200 ml/min flow rate of sampling. Sinus NO was measured as ppb through 200 ml/min flow rate of sampling. Sinus volumes and mucosal surface area of each sinus are calculated from a 3D CT model of the sinuses by digitization software (iplanet).

Subjects were instructed to have a light breakfast and to avoid caffeinated beverages or food and to present at 8:00 AM on the study day. After registration subjects were brought to the lab to acclimatize. A baseline nasal NO measurement was
performed as described previously [45,48]. Topical spray mix of 2 cc (15 cc of oxymetazoline with 2 cc of 1% lidocaine) was applied. Ten minutes later a sterile injection of 1% lidocaine plus 1/100000 epinephrine solution into the uncinate, lateral nasal wall submucosa and attachment of middle turbinate to the lateral nasal wall were performed. A total of 7 cc was used. After 20 min, based on the pre-determined sinus of interest, with the help of either a 0° or a 30° Hopkins telescope, a Relieva Flex S-0, F-70C or M-110 guide (Acclarent, Inc) attached to a Sidekick (Acclarent Inc) (a handle) was used to approach the sinus ostium. Relieva Luma Light probe (LUMA) (Acclarent Inc) is used to enter the sinus through the guide, providing verification of sinus entry through illumination. A sterile polyethylene catheter tube (PEC) (Clay Adams, Intramedic) with an outer diameter of 1.27 mm was introduced into the sinus over the LUMA. The LUMA was carefully withdrawn and the PEC was secured to the facial skin with tape. All procedures were performed with sterile instruments. The NO analyzer collection line was attached to the polyethylene tubing through a disposable liquids filter to prevent contamination of analyzer sampling line.

3. Results

1. The office setup for endoscopic procedures is ideally suited for individual catheterization of paranasal sinuses (Fig. 1). Equipment frequently used for office nasal endoscopy and postoperative debridement such as suctioners, forceps (Hartmann, Alligator) are sufficient for the placement of catheters into the sinuses. Video screen and camera as well as 0° and 30° Hopkins telescopes are used. A second light source for the LUMA is necessary. Sterile surgical gown and gloves should be worn by all involved.

2. Topical anesthesia (Lidocaine or Pontocaine) only is inadequate for pain relief. Submucosal local anesthetic (LA) infiltration of the uncinate, middle turbinate, septum and lateral nasal wall is required.

3. Catheter placement goals should be flexible and practical. Trying to place catheters into all major paranasal sinuses on one side is generally not feasible and should not be attempted. Each additional catheter placement carries the risk of pulling out previously placed catheters during the process. If two sinuses are entered successfully on one side, a third attempt on the same side should be avoided and the contralateral sinuses should be addressed.

4. Timing, skillful and swift handling is essential and possible only with help. As the effects of the LA wear off, delicate maneuvers to place and keep catheters in place become more difficult. Ideally, all catheterization should be performed within the hour after the LA infiltration.

5. In many cases, an irregular nasal septum prevents comfortable access to one or more sinus. To prevent damage to mucosa and risk synchia formation, one should pick the easiest sinus access and the smallest guide with the proper tip angle. Guides should be introduced with their tips turned as close to ostial orientation as possible to prevent large diameter turn maneuvers within the middle meatus or frontal recess.

6. Pediatric-sized guides are best for non-traumatic access. Suction-enabled new-generation guides offer superior visualization. For the sphenoid sinus, the straight guide can be used.

7. Label and secure each catheter with mastisol or similar facilitator and tape to skin, to prevent the catheter from coiling out of the sinus (Fig. 2).

8. After catheter placement, have patient relax and rest until the effects of topical and local anesthetics wear off. Prepare to suction and clean subjects’ nasal passages frequently as there will be a rebound congestion and increase in secretions causing discomfort to subject. The subject is not allowed to touch his/her nose or even raise hands above chest level since inadvertent displacement of catheters will occur when subjects have an itchy nose.

9. Check each PEC’s position with the LUMA after placement and again before NO measurements. Check to ensure that there is no blood in the catheters and none enters the PEC during sampling; using a luer lock syringe, gently suction all hemorrhagic material before attaching PEC to sampling line of the NO analyzer. Any residual hemoglobin will interfere with NO measurements.

10. During sampling, check sampling pressures and flow rates frequently since contact between the catheter and sinus mucosa will obliterate the catheter.

11. Use a sharp knife to cut polyethylene catheters (PEC) to 25-cm size, and mark the 24-cm level from the tip of the

Fig. 1 – Procedure setup and assistant’s position who is feeding the LUMA and PEC.
12. NO measurements can be repeated several times, the sampling line can be left attached to any catheter any length of time and several maneuvers can be performed during each sampling.

13. If a nasal passage is to be isolated from the lower airway with a Foley catheter, this is best done toward the end of sampling since this procedure may cause mild discomfort and reflex vasomotor increase in secretions. A pediatric 8Fr Foley is introduced easily through the nostril. Once the balloon is observed in the oropharynx, gentle retraction and inflation of the balloon with 1.5–2 ml of saline will place the balloon at the choana but will not completely seal it. The protruding end of the Foley will need to have gentle traction applied to keep a tight choanal seal. This can be accomplished by hanging a small weight (a water-filled 200-ml plastic bottle) through a pulley/line that pulls the Foley at the same vector angle as the nasal floor has. The hanging weight is tolerated for a short time during which isolation data can be obtained. If research includes sinus lavage, this technique prevents loss of lavage fluid sample through the choana and ensures improved recovery of lavage fluids.

14. A patent nasolacrimal duct (NLD) may introduce variation during blinking especially when the choana is sealed and steady NO measurements from the sinus are expected. This occurred in one subject: each time she blinked, we had a measurable drop in the maxillary sinus NO level which otherwise was at a steady plateau. Similar variations can be observed during swallowing if the choanal seal is not fit tight enough.

15. Once the PEC is in the sinus, a delicate sequence of maneuvers under endoscopic control has to be performed. An assistant is required. First, the LUMA is
gently withdrawn while keeping the PEC in place. Next, an alligator forceps is used to gently grab the PEC at the point as it exits the tip of the guide and enters the sinus. To do this, the guide needs to be withdrawn a few millimeters. The alligator forceps can easily crimp and damage the soft PEC, therefore, the PEC needs to be grabbed gently. It helps to use an ear alligator forceps with smooth blades. Once crimped, the PEC is rendered useless and needs replaced. Next, the guide is pulled while keeping the PEC in place with the alligator. Once the guide is removed, the alligator is withdrawn. Any crimping on the PEC should be visualized. LUMA is carefully introduced through the PEC to verify its position. If LUMA can’t be advanced easily, the PEC is most likely crimped and needs replaced.

16. While NO is measured, nasal breathing produces Venturi effect at the sinusal interface and pulls NO out of the sinuses causing a rapid decrease during inspiration and a slower increase during expirium creating a spiked waveform [57] (Fig. 4).

17. None of the subjects reported adverse effects after the procedure. One subject with extremely low pain threshold could not cooperate and was cancelled. It is important to detect these subjects during initial endoscopic examinations. Any hint of compromised cooperation (high anxiety, low pain threshold, jumpy and hypermobile subject, reflexive, defensive hand motions and posture, etc.) during screening examinations should prompt dismissal. Another subject presented without having breakfast and had hypoglycemic hypotension although she was never diagnosed with diabetes. She was cancelled for the day but was invited 2 weeks later and participated without problems.

18. Detailed explanation of the procedures step by step is very helpful. Subjects like to be informed of the immediate next step during the procedures. Surprise injections or sudden inflation of a choanal Foley balloon may lead to poor cooperation later on.

19. Allowing subjects to listen to music of their choice works well especially during the slower sections of the study such as acclimatization, measurement of long-term changes in sinus NO etc.

20. Although the ostial diameter is usually much larger than the PEC diameter, individual variations are possible and can be detected on a CT scan. If the ostium is very small, the PEC can obstruct it and sampling sinus air will create a vacuum in the sinus. This is evident by a rapidly dropping sampling flow rate which should be carefully monitored, and also sinus pain experienced by the subject. Pain can be intense, and sampling should be stopped.

4. Conclusion

Differential sinus NO measurements through catheter placement into individual sinuses are feasible. The technique provides a valuable human study model for research on sinonasal aerodynamics, sinonasal gas exchange, changes in pressure, NO or CO2 levels in the sinuses in various sinus disease models and rhinitis, as well as for pre- and postoperative comparison studies. This model has a great potential to provide the basis for our improved understanding of sinus function in health and disease states.

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REFERENCES


Beckman JS, Beckman TW, Chen J, et al. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci USA 1990;87:1620–4.


Rabinovitch N, Zhang L, Gelfand EW. Elevated exhaled nitric oxide levels are associated with daily lung function declines and increased medication usage in urban children with moderate to severe asthma despite use of inhaled corticosteroids and long acting beta agonists. Poster at American Academy of Allergy, Asthma and Immunology 2007.


