

VIII. Nasal NO Measurements

Just as exhaled NO has been studied as a marker of airway inflammation, nasal NO has been studied as a marker of nasal inflammation. [Alving K *et al.* 1993] In the nose, NO is thought to play a role in host defence through its own antimicrobial and antiviral activity and by up-regulating ciliary motility. [Djupestrand PG *et al.* 2001; Kim JW *et al.* 2001] Most of the NO in nasal air is derived from epithelial cells in the paranasal sinuses, with concentrations in the sinuses being close to the maximum allowed atmospheric levels. [Lundberg JO *et al.* 1995; Lundberg JO *et al.* 1994a] It is thought that the high levels of NO in the sinuses play a role in maintaining sinus sterility.

Several techniques have been described, including tidal breathing and fixed-flow techniques. A nasal NO method is available for the NIOX® system and this has been shown to provide reproducible results. Kharitonov and colleagues used this method in adults and children with and without asthma, and found the coefficient of variation to be low at 12.5% (95% confidence intervals 11.0–14.7%). This value was even lower in adults (7.7%) (Figure VIII.1). [Kharitonov SA *et al.* 2005] The mean number of attempts required to achieve three appropriate measurements was 5.4 and this did not differ significantly between adults and children. The authors concluded that this technique was reliable and acceptable to patients for the measurement of nasal NO.

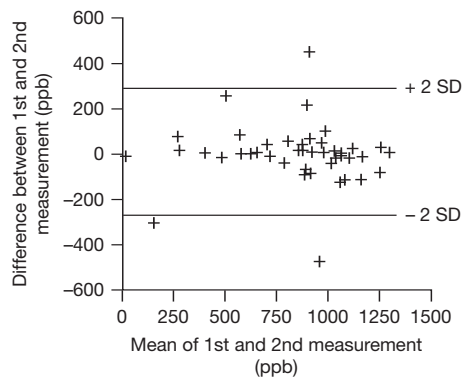


Figure VIII.1. Bland–Altman analysis for the repeatability of nasal NO (difference was computed as the first–second measurement. If first or second measurement was missing the third measurement was used. Means computed in the same way. The mean difference was 6.53 ppb and the SD of the difference was 125.5 ppb. This yields the “limits of agreement” $-6.53 - [2 \times 125.5] = -257$ ppb and $-6.53 + [2 \times 125.5] = 245$ ppb) [Kharitonov SA *et al.* 2005]

Like exhaled NO, guidelines are available for nasal NO measurements. [2005; Barreto M *et al.* 2005] Generally, in healthy individuals nasal NO levels are much higher than exhaled NO levels, with values over 100 and up to 2,000 ppb using various techniques (Table VIII.1). [Cervin A *et al.* 2002; Dotsch J *et al.* 1996; Grasemann H *et al.* 1999; Horvath I *et al.* 2003; Karadag B *et al.* 1999; Lefevre L *et al.* 2000; Lindberg S *et al.* 1997; Loukides S *et al.* 1998; Narang I *et al.* 2002; Noone PG *et al.* 2004; Tornberg DC *et al.* 2003; Wodehouse T *et al.* 2003] Stark *et al.* demonstrated that nasal NO levels exhibit diurnal variation, with lower values in the morning compared with the afternoon. [Stark H *et al.* 2007]

Table VIII.1. Nasal NO levels in healthy individuals.

Author, year	Nasal NO level (ppb)
Tornberg <i>et al.</i> , 2003	177 ± 17
Cervin <i>et al.</i> , 2002	164 ± 10.3
Dotsch <i>et al.</i> , 1996	96 ± 47
Lindberg <i>et al.</i> , 1997	233.2 ± 66.8
Grasemann <i>et al.</i> , 1999	1195 ± 618.2
Horváth <i>et al.</i> , 2003	663 ± 255
Karadag <i>et al.</i> , 1999	553 ± 330
Loukides <i>et al.</i> , 1998	826 ± 37
Narang <i>et al.</i> , 2002	716 ± 260
Noone <i>et al.</i> , 2004	752 ± 248
Wodehouse <i>et al.</i> , 2003	759 ± 145.8

Cervin *et al.* have shown nasal NO measurements to decrease within 10 minutes after nasal challenge with histamine and after challenge with the vasoconstrictor oxymethazoline in healthy non-allergic subjects. [Cervin A *et al.* 2002] Both plasma exudation and nasal blockade increased within 10 minutes of the challenge, indicating that the different signs of airway inflammation are not directly linked and may reflect different aspects of nasal mucosal inflammation. Tornberg *et al.* also showed that nasal NO levels are significantly higher in patients during anaesthesia (315 ± 34 ppb) than while awake (177 ± 17 ppb; $p = 0.01$). [Tornberg DC *et al.* 2003] A study carried out on premature infants (median gestational age of 27 weeks), showed that nasal NO could be detected directly from the nasal space using a chemiluminescence analyser. [Williams O *et al.* 2003] Lower airway NO was also sampled from a catheter positioned so that its tip lay at the lower end of the endotracheal tube. Nasal NO levels were higher than lower airway NO levels, even on the first day after birth, showing that care must be taken to avoid contamination with nasal NO when assessing lower airways accurately. Nasal and lower airway NO levels did not correlate significantly with gestational age, but lower airway NO levels correlated with postnatal age ($r = 0.86$, $p = 0.014$).

8.1. Nasal Humming

Although attempts to standardize nasal NO measurements have been made, detecting alterations in nasal output is limited by the high background NO levels in the upper airways originating from several sources in the nose and sinuses. In particular, the paranasal sinuses seem to be major sources of NO and local concentrations can reach well over 20 ppm. [Lundberg JO *et al.* 1995] Nasal humming has been shown to speed up the exchange of air between sinuses and the nasal cavity thereby dramatically increasing nasal NO output. [Maniscalco M *et al.* 2003b; Weitzberg E and Lundberg JO 2002]

Maniscalco and colleagues showed that during repeated humming manoeuvres an initial NO peak is observed, followed by a progressive decline (Figure VIII.2). [Maniscalco M *et al.* 2003b] This is probably because most sinus NO has been washed out. The same group also showed in a different study that nasal NO levels measured immediately after repeated humming manoeuvres are consistently lower and more reproducible than nasal NO levels measured after a period of silence or free speaking, probably due to the sinus NO contribution being

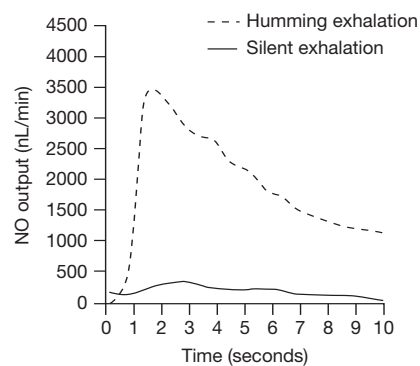


Figure VIII.2. During nasal humming an initial NO peak is observed followed by a progressive decline [Maniscalco M *et al.* 2003b]

temporarily decreased and thereby removing the variability in NO measurements conferred by nasal NO. [Maniscalco M *et al.* 2003a] Mean nasal NO output (95% CI) after a period of silence/free speaking was 231 nL/min (range 178–284 nL/min) in healthy volunteers, 434 nL/min (range 347–522 nL/min) in patients with allergic rhinitis ($p < 0.001$), and 262 nL/min (163–361 nL/min) in patients with allergic nasal polyposis. The authors concluded that repeated humming manoeuvres could be useful to better distinguish nasal mucosal NO output from that of the paranasal sinuses. A mathematical model of the changes in NO output during humming has been published. [Menzel L *et al.* 2005] Granqvist *et al.* investigated the acoustic phenomena responsible for the evacuation of NO from the sinuses using a tube model. [Granqvist S *et al.* 2006] Their findings suggested that alternating pressure in the nasal cavity forces the air plug in the ostium of the paranasal sinus resonators to vibrate, thus expelling NO, which is transported to free air by the exhalatory air stream.

Remarkably, the increased NO output achieved through humming may have a therapeutic role. A case report suggested that strong humming could eliminate the symptoms of chronic rhinosinusitis (a condition caused by fungal infection) and the authors speculate that this could be due to the antifungal properties of high NO levels. [Eby GA 2006]

8.2. Rhinitis

Several studies have indicated that nasal NO levels are increased in patients with allergic rhinitis. [Arnal JF *et al.* 1997; Baraldi E *et al.* 1998a; Eby GA 2006; Kharitonov SA *et al.* 1997b] For example, Kharitonov and co-workers reported nasal NO levels to be 1527 ± 87 ppb in untreated patients with allergic rhinitis, whereas levels of 996 ± 39 ppb were found in healthy controls. [Kharitonov SA *et al.* 1997b] In addition, nasal NO levels were lower in those patients who received nasal corticosteroid treatment. Profita *et al.* reported that nasal NO was increased in allergic rhinitis and asthma compared with healthy subjects. [Profita M *et al.* 2006] In allergic rhinitis, there was a positive correlation between nasal NO and IL-5 levels and with duration of disease. Similar results have been reported by other groups. [Baraldi E *et al.* 1998a; Sandrini A *et al.* 2003b]

However, other groups have shown no significant difference in nasal NO levels between patients with rhinitis or similar nasal symptoms and healthy individuals. [Olin AC *et al.* 1998; Smith AD *et al.* 2005a; Moody A *et al.* 2006] For example, Moody *et al.* investigated nasal NO levels in 38 patients with perennial rhinitis. [Moody A *et al.* 2006] The results indicated that nasal NO levels are not elevated in these individuals compared with controls and, furthermore, there was no correlation between nasal NO levels and symptoms of the condition. In addition, some authors have found no effect of treatment on nasal NO levels in patients with allergic rhinitis. [Wilson AM *et al.* 2000] Whether nasal NO levels are increased in rhinitis is still open to debate. Given that most nasal NO originates in the sinuses, a more conclusive study regarding rhinitis would involve measurement of nasal NO that excluded sinus NO. Currently no technique exists for this measurement, although measurements by nasal humming may be an option. It has been shown that nasal epithelial cells from patients with rhinitis express higher levels of iNOS than cells from healthy individuals, suggesting the presence of higher NO levels in affected patients. [Takeno S *et al.* 2001]

8.3. Rhinovirus Infection

Rhinovirus infections are associated with unpleasant symptoms and exacerbate asthma and COPD. NO has potent antiviral properties and thus may have a role in host defence. If this is the case, one would expect nasal NO levels to be increased in individuals infected with rhinovirus.

Sanders and co-workers investigated nasal NO levels in six volunteers who were infected with rhinovirus-16. [Sanders SP *et al.* 2004] NO levels increased significantly and were associated with increased expression of iNOS mRNA in nasal scrapings. Furthermore, the increase in nasal NO correlated inversely with symptom scores, possibly indicating an antiviral role. The group had previously shown that rhinovirus increased iNOS mRNA expression *in vitro* and in humans. [Sanders SP *et al.* 2001]

8.4. Primary Ciliary Dyskinesia

An area where nasal NO measurements prove particularly useful is in the diagnosis of PCD. Karadag and colleagues reported that nasal NO levels in patients with PCD were much lower than in healthy individuals (97 ppb vs. 664 ppb). [Karadag B *et al.* 1999] Interestingly, patients who had PCD with a lack of inner dynein arms only had much higher NO levels (869 ppb) than the other affected patients. Others have also confirmed that PCD is associated with extremely low levels of nasal NO [Lundberg JO *et al.* 1994b] and these low levels seem highly predictive of the disease.

Narang and colleagues studied exhaled and nasal NO in 102 children attending a respiratory clinic and compared their results with those of 53 healthy controls. [Narang I *et al.* 2002] The children with respiratory disease included patients with PCD, asthma, CF and non-CF bronchiectasis. Nasal air was sampled from one nostril during breath-hold, using a sampling rate of 250 mL/min. Nasal NO levels were significantly lower in PCD patients than in all the other groups. Median values were 60.3, 533.6, 491.3, and 716.0 ppb in children with PCD, bronchiectasis, CF, and controls, respectively ($p < 0.05$). Only one patient with PCD had a nasal NO level greater than 250 ppb, and 80% had nasal NO levels of less than 100 ppb. There was some overlap with patients with CF and bronchiectasis, but the authors calculated that nasal NO levels of less than 250 ppb had a positive predictive value of 83% and a negative predictive value of 97% of identifying patients with PCD. Sensitivity and specificity for PCD at various cut-off points for nasal NO are shown in Figure VIII.3. In another study NO levels < 105 ppb had a positive predictive value of 89% and a negative predictive value of 100% for predicting PCD. [Corbelli R *et al.* 2004] Horváth and colleagues found similar results in their study. [Horvath I *et al.* 2003] They measured exhaled and nasal NO levels in bronchiectatic patients with PCD ($n = 14$), non-PCD bronchiectatic patients with CF ($n = 20$) and without CF ($n = 31$), and healthy volunteers ($n = 37$). Nasal NO levels were significantly lower in PCD patients than in any other group (PCD: 54.5 [5.0–269] ppb; non-PCD bronchiectasis without CF: 680 [310–1000] ppb; non-PCD bronchiectasis with CF: 343 [30–997] ppb; control: 663 [322–1343] ppb). Exhaled NO levels followed the same pattern, but levels were not lower than in bronchiectatic patients with CF. PCD can be difficult to diagnose and thus nasal NO measurements are useful in diagnosing the condition, [Bush A 2000; Bush A *et al.* 1998] particularly as the test is simple to perform.

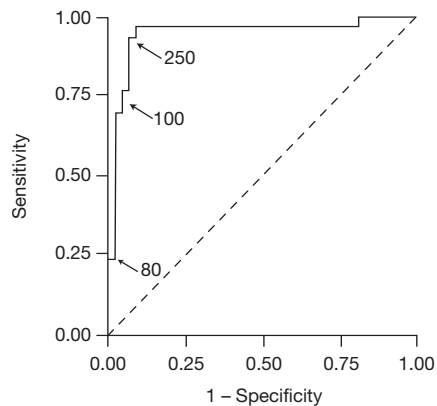


Figure VIII.3. Receiver-operator characteristic curve showing the value of various nasal NO concentrations in the diagnosis of PCD [Narang I *et al.* 2002]

8.5. Cystic Fibrosis

As with exhaled NO measurements, nasal NO levels are lower than normal in individuals with CF, although not to the same extent as seen in PCD. [Lundberg JO *et al.* 1996a; Thomas SR *et al.* 2000; Narang I *et al.* 2002] Nevertheless, nasal NO measurements may prove helpful in the differential diagnosis of this condition.

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