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Measurement of Exhaled Nitric Oxide Using End Tidal Value during Normal Breathing

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Abstract

Increased nitric oxide (NO) production in the expired air has been associated with a number of disease conditions and may reflect the severity of inflammation in the lungs. Measurement of exhaled NO concentration has been proposed as a novel clinical tool for assessing airway inflammation and response to drug therapy. In spite of international guidelines aimed at standardizing the measurement of exhaled NO, clinicians remain skeptical due to the necessity of a significant degree of subject collaboration required by the procedure to obtain meaningful measurements. We hypothesized that exhaled NO concentration may be best measured by using end tidal NO concentration during a breath by breath monitoring. We tested laboratory staff and monitored their NO concentration online using a fast response chemiluminescence NO analyzer during normal breathing at rest for 5 minutes. First we confirmed that the NO analyzer was adequately fast to record fully the swings during normal breathing. We then compared the average end tidal NO value (NOet) from 6 to 10 breaths, to the NO value obtained from the plateau from a single slow vital capacity maneuver as recommended by the ATS guidelines (NOplat). End of exhalation was identified using end tidal carbon dioxide measurement. NOet while breathing room air was 24.3 ± 4.2 (SE) ppb and was not significantly different compared to 22.4 ± 2.8 (SE) ppb using NOplat. Additionally breathing high level of NO of up to 88 ppb did not affect either NOet or NOplat significantly suggesting that both measurements are independent of inhaled NO concentration. NO value at end of exhalation was very reproducible from breath to breath and did not require any special effort on the part of the subject. We recommend using NOet measurement instead of the NOplat value that is highly dependent on the exhalation rate and cooperation of the subject. NOet is much easier to record in children, elderly and patients with respiratory disease. The main requirement for measuring NOet is to have a fast response NO analyzer to record the full swings in NO concentration during normal breathing and to have a way of identifying end exhalation.

Keywords: Exhaled nitric oxide; Tidal breathing; Single breath; Human

Introduction

Nitric oxide (NO) has been detected in the exhaled gas of humans and animals [1]. The level of NO in exhaled gas has been shown to increase in certain states of lung inflammation [2,3] and has been suggested as a new clinical test [4]. Indeed, Exhaled NO has been widely used in asthma as a measure of severity of inflammation in the lungs [5] and in the management during drug therapy [6,7]. NO is produced by many cells in the lung and is released into the airspace including airways and alveoli [8-10]. Having both alveolar and airway components, the manner used to measure exhaled NO can affect the outcome and continues to be a subject for debate [11]. Recognizing that the measurement is flow dependent, current guidelines recommend a slow vital capacity maneuver at a fixed flow rate of 50 ml/sec against a resistance [12-14] which is a compromise between measurement sensitivity and patient comfort. While this method has been reluctantly adopted by many, it may be biased against detection of NO in the alveolar space and has been shown to be difficult to measure in some situations especially those involving children or uncooperative subjects [15].

The guidelines were recommended to create uniformity in measuring and reporting exhaled NO and not to preclude other valid methods for measuring NO. The guidelines correctly emphasized that the recommendations should not necessarily invalidate other sensible and reasonable methods. Whether these recommendations have helped in bringing about some uniformity and more reliable application of NO measurement is not clear but disagreements remain after so many years [16-19]. Furthermore, the guidelines have justifiably influenced the industry and some companies have manufactured NO analyzers narrowly based on the guidelines, potentially hindering the development of better methods for NO measurement. While some clinicians remain skeptical about using NO measurement [16-19], others believe the measurement is very useful and helps with the management of asthma [6,20-22]. More importantly, the changes in NO concentration in exhaled air in lung diseases can be small and subtle and can be easily overshadowed by methodological errors, such variations as in flow rate or patient's cooperation. Therefore it is prudent that we use a reliable method to measure exhaled NO as a meaningful clinical tool. Our goal was to show that exhaled NO can be easily and more reliably assessed using end tidal NO concentration.

Material and Methods

Males and females volunteers (n=19) ranging from 27 to 52 yrs in age, and in good health were invited to participate in the study. The volunteers were laboratory personnel. The study was approved by the Institutional Review Board and a consent form was signed by all volunteers. None of the subjects had any symptoms of respiratory tract infection and none were habitual smokers.

Protocol of the study

Figure 1 shows a diagram of the set up that was used for the study. The subject was seated in a comfortable chair, and the mouthpiece was

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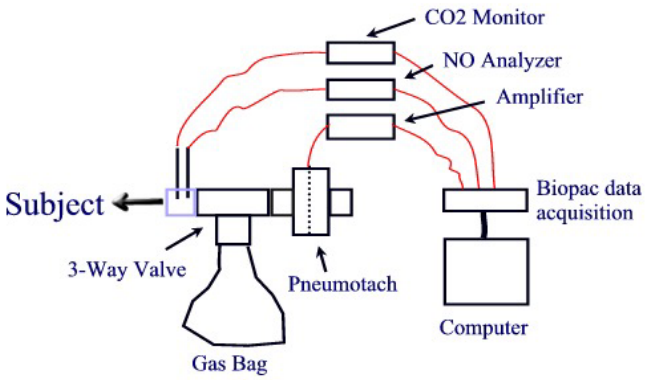

Figure 1: Experimental set up that was used. The 3 way valve allowed the subject to inhale from the bag and exhale through the pneumotach.

adjusted for easy reach by the subject. The subject breathed through the mouth (with a nose clip) through a disposable mouthpiece connected to a 3 way valve and a pneumotach (Biopac Systems TSD 117). This set up allowed inhalation from the bag and expiration through the pneumotach while sampling gas continuously from side ports near the mouthpiece for CO2 and NO measurement. NO was measured using a fast response chemilumiscenece NO analyzer (Sievers 270B, Boulder, CO) and CO2 was measure using a CO2 monitor (Datex Ohmeda, Madison, WI). The inspiratory limb of the 3 way valve was either open to room air or was connected to one of the bags that contained a known concentration of NO (up to 88 ppb). Gas was sampled into the NO analyzer at 400 ml/min. The signals from the NO analyzer and CO2 monitor as well as the pneumotach were fed into a computer for continuous breath by breath recording and observation. The analog signals were digitized at 10 HZ aligned and stored continuously on a Macintosh computer using data acquisition software (MP100 BIOPAC Systems, Goleta, CA). The output from the NO analyzer was recorded continuously and displayed as ppb. The average NO concentration from 0.5 to 1 sec of data near end expiration was measured and the values from a few breaths (5 -10 breaths) was used as end tidal NO concentration (NOet). Calibration of the NO analyzer was performed daily and zero was verified repeatedly during the experiment. Room air NO was also recorded repeatedly during the experiment. The linearity of the NO analyzer was verified by diluting NO sample serially 4 times down to 10 ppb. One group of subjects were studied to compare end tidal NO while breathing different levels of NO, and a second group were studied to compare end tidal NO with the plateau NO value derived from the single breath method as suggested by the ATS/ERS guidelines. The two groups were studied on separate days. Some of the subjects that were used in group one, were also used in group two.

In the first group the subjects were asked to breathe normally with the 3 way valve open to room air. Once the subject was relaxed, recording began for about 5 to 10 min. Breathing was then switched to the next bag with the higher concentration for another 5 to 10 min, and so on with the other NO bag mixtures (described below). This part of the study was designed to test the adequacy of the response time of the NO analyzer during normal breathing. In the second group, the subjects were asked to breathe room air for 5 minutes, at the end of which the subject was asked to take a deep breath, and exhale slowly at a rate of 50 ml/s until all the air is expelled. The plateau near the end of expiration was used to measure NO concentration (this maneuver was done to reproduce the procedure that was recommended by the ATS guidelines). After a short break, the procedure was repeated, while breathing from the bags with the higher NO concentrations. This was repeated for the other bags containing higher NO concentrations. The single slow expired breath maneuver was done only once in each case to record plateau NO level from a single breath (NOplat), no resistance was used, and a nose clip was present.

The analyzer was calibrated daily by introducing air with zero NO from a gas cylinder and from a mixed gas bag containing 248 ppb which was prepared from a certified gas tank containing 1424 ppb NO in pure air (MG Industries, Valley Forge, PA). The gas concentration was diluted to 248 ppb for calibration. The gas bags were prepared by filling them with a known volume of air from a tank of medical grade compressed air using a 5 L syringe. A small volume of the certified nitric oxide gas mixture was added using a 50 cc glass syringe. There were 3 or 4 bags with different levels of NO of up to 80 ppb ready for use. Room air NO concentration was monitored daily, and when it exceeded 5 ppb, the subject breathed from a bag containing room air with zero NO from a compressed gas tank (was necessary in 1 of the 13 subjects). The bags were prepared shortly before the experiment started. The bags contained NO with a calculated concentration of 0, 24.9, 47.8, 80.3 ppb NO. The concentration of NO in the bag was measured offline after it was prepared and was usually within 5% from the calculated value. We selected these concentrations because they are safe, and represent the range of variations in NO in ambient air in our facility.

Statistical analysis of the results was accomplished using ANOVA for repeated measures and post hoc t-test (Boneforroni test). All results are expressed as means ± SEM. Relationship between NOet and NOplat, was also examined using a linear regression analysis. P values of <0.05 were considered significant.

Results

A typical recording from one subject in the first group with the different levels of inhaled NO (NOi) is shown in Figure 2. Note the breath to breath consistency in NO at end expiration (NOet) during normal breathing regardless of the level of inhaled NO. End tidal NO was nearly identical from one breath to another (arrows). Furthermore, peak NO during inspiration was very close to NO in the bag suggesting that the response of the analyzer was adequate to record with accuracy inhaled and exhaled NO concentration during normal breathing. End tidal NO (arrows) remained unchanged while breathing different levels of NO. On a tracing like this, it would have been difficult to be certain which peak is end expiration without the CO2 tracing being available simultaneously.

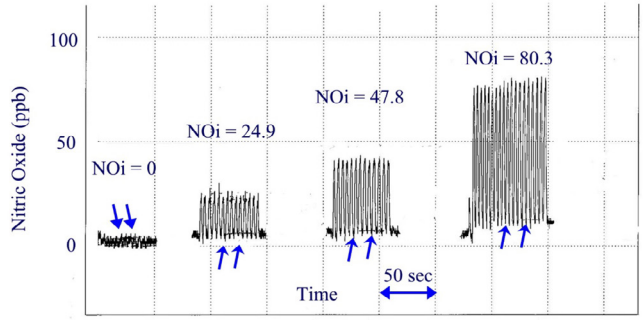

Figure 2: Tracing of NO during normal breathing. Inhaled NO in the bag is indicated (NOi) and arrows point to the tracing at end expiration. With room air (NOi=0) end expiration is on the top of the tracing. In contrast, during breathing high NO mixture, end expiration is near the bottom of the tracings. Note the consistent value at end expiration for all breaths.

An expanded view of few breaths showing CO2 and NO is shown in Figure 3. Regardless of the level of inhaled NO or the shape of NO peak at the start of expiration, end tidal NO concentration, identified from CO2 tracing was easily quantified. In this example, the subject had very low end tidal NO. CO2 recording was helpful to identify a stretch (0.5 to 1 sec) of data that could be averaged and would represent end tidal NO value for each breath. Peak value during inspiration was usually within 5% of the bag calculated value, and was somewhat difficult to read because there was no delay or a plateau compared to end expiration where NO level was flat for 1 to 2 seconds. The shape of NO tracing varied among subjects while breathing room air, some exhibited an early peak that may have been related to contamination from nasal NO. The concentration at end expiration could be easily identified and averaged over 0.5 to 1 second of data with the aid of the CO2 tracing. The values from 5 to 10 breaths were averaged to represent NOet for the subject.

There was a wide range in NOet values (0.2 ppb to 53 ppb) among the subjects. In the first group of subjects, average NOet was 15.4 ± 2.2 ppb (± SE) when the subjects breathed room air, and did not change significantly when they breathed from the bags containing higher NO concentrations (Figure 4). While breathing from the bag with high NO, NOet was 15.3 ± 2.1ppb, 16.3 ± 2.0 ppb, and 16.5 ± 1.8 ppb respectively and were not significantly different from each other (P>0.4). End inspired NO was not significantly different from the NO in the bag (P>0.1), suggesting that the NO analyzer had an adequate response time to record accurately the full swings in NO concentration during normal breathing.

In the second group of subjects, NOet and in NOplat, also exhibited a wide range among the subjects, 0.2 to 53 ppb and 7 to 43 ppb respectively. As stated earlier, some of the subjects used in group 1 were

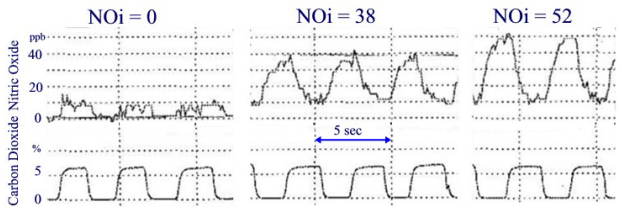

Figure 3: Expanded view of the NO tracings along with CO_2 tracing. End tidal NO was the average over 1 to 2 seconds during the time when CO_2 was plateau at peak value. The average of 5 to 10 breaths was used as NOet. Inhaled NO (NOi) is given during each segment of the tracing.

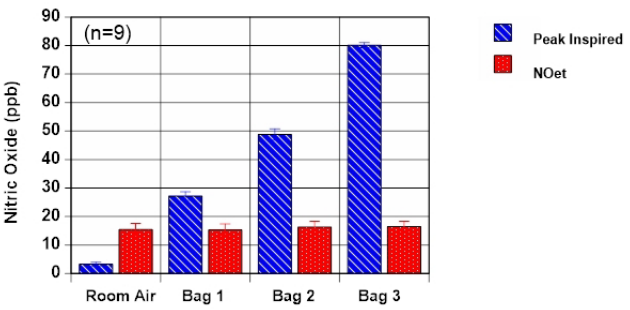

Figure 4: Average end tidal NO (NOet) during breathing room air and breathing high NO mixture. Peak NO during inspiration was very close to NO concentration in the bag. NOet did not change and was independent of inhaled NO (P>0.4).

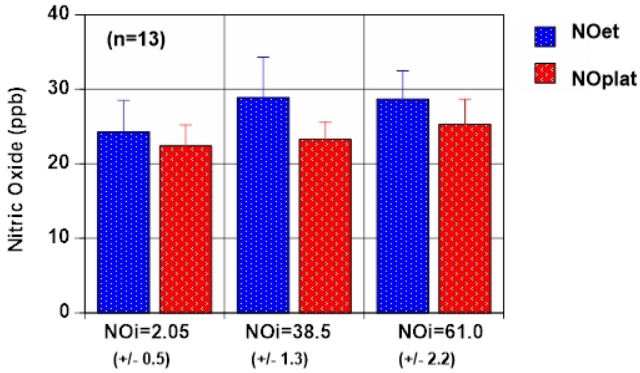

Figure 5: NOet *versus* NOplat as measured using the single breath exhalation according to the ATS guidelines. There were small differences that were not significant (P>0.4). Both NOet and NOplat were independent of inhaled NO (P>0.4).

also inlcuded in this group. Average NOet vs NOplat are compared in Figure 5. This group of subjects breathed either room air (NOi=2.1 ± 0.5 ppb) or from a bag containing 38.5 ± 1.3 ppb and a bag containing 61.2 ± 2.2 ppb. NOet was 24.3 ± 4.2, 28.9 ± 5.4, and 28.7 ± 3.8 ppb respectively while NOplat was 22.4 ± 2.8, 23.3 ± 2.3. 25.3 ± 3.4 ppb respectively. In either case the measured NO was independent of the inhaled NO concentration. Furthermore, NO measured with the two methods were not significantly different (P>0.4) from each other. The changes in NOet or NOplat while breathing high NO mixture were not statistically significant (P>0.4). The relationship between NOet and NOplat from linear regression analysis through the origin was NOplat=0.78NOet (R2=0.85).

Discussion

In this study we found that exhaled NO measured from NO value at end expiration during normal tidal breathing (NOet) was not significantly different from that measured from the single slow vital capacity maneuver [13,14] but was much easier to perform. Measurement of NOet required no special instructions and no extra effort from the subject. The subjects were simply asked to breathe room air normally for a few minutes. Furthermore, NOet was independent of inhaled NO within the normal environmental range suggesting that day to day variations in ambient NO do not influence NOet measurement. Therefore, we suggest that monitoring breath by breath NO and using the average NOet concentration is preferred over NOplat, from the single breath method recommended by the ATS guidelines [13,14]. The single slow vital capacity maneuver was done using exhalation rate in the range that was recommended by the ATS guideline, however rate of exhalation can affect the measured NO value significantly. Thus the agreement between the two measured values is valid only for a specific flow rate that was used during the single slow full capacity maneuver. Although the limitations maybe different, both methods are likely to provide NO concentration representing NO production from the lungs that would have similar clinical and physiological implications. The main requirement for measuring NOet is having a NO analyzer with a response time fast enough to record swings in NO during tidal breathing, and a marker for identifying the end expiration time. The response time of our NO analyzer according to the manufacturer specification is 67 msec to 90% full scale, which makes it very suitable for measuring end tidal NO during normal tidal breathing.

The main thrust of the ATS guidelines was to encourage uniformity

in measuring and reporting exhaled NO values that took into account online measurement, flow dependence and potential nasal contamination [14]. While recognizing that NO measurement was highly dependent on flow rate used during exhalation, the slow vital capacity maneuver against a fixed resistance at a moderate flow rate, that was recommended, achieved an acceptable compromise while making the measurement easier. As such, children and non-collaborative subjects are yet often unable to perform these maneuvers [15] in spite of numerous visual feedback systems, thus limiting the reliability of the method. Equally, this maneuver, biased towards measurement of airway derived NO, has limited the widespread application of the measurement for conditions involving NO derived from a peripheral compartment such as the alveolar space. Complex procedures such as measurements at variable rates have been suggested [23,24] which however made it even more difficult and lengthy. The single slow breath maneuver, requires taking a slow deep breath over 2 to 3 seconds. This may allow more NO in the alveolar space to diffuse into the blood, (to some extent similar to a breath hold) thus causing the measurement to underestimate alveolar NO concentration. Thus the slow full capacity maneuver against a resistance is somewhat subject to errors and maybe too difficult to perform especially for children and elderly. It also may be burdensome for patients with pulmonary disease, especially if they have to repeat the maneuver 2 or 3 times. NOet measurement avoids such problems and in our opinion, besides being easier, is a more reliable method for measuring exhaled NO for clinical purposes. Indeed, end tidal CO2 and O2 have been the gold standard for measuring gas concentration in the lungs for many years. End tidal NO value was stable over many breaths and much easier to identify. When necessary, changes in minute ventilation, which can affect exhaled NO concentration, can be accounted for by calculating NO production [25]. The effect of nasal contamination [26] on NOet measurement is likely to be very small during normal breathing because most of the NO would have been washed away early during expiration, and can be further minimized by applying a small negative pressure at the nose [27]. Nasal contamination may be more significant in other situations such as when measuring NO in mixed exhaled gas, measuring peak NO, or breath holding where nasal NO can accumulate in the upper airways.

The ATS guidelines attempted to create a uniform method for measuring and reporting exhaled NO, however, investigators remain reluctant about the procedure, and have looked for other ways to measure exhaled NO [28-30]. Some studies compared the single breath measurement with measurement from mixed expired air during tidal breathing [15,29,31]. Mixed expired samples may be highly affected by NO in dead space (physiological and anatomical) or contamination by NO derived from ambient or nasal NO. Most studies found exhaled NO measured by the single breath method to be unaffected by inhaled/environmental NO [32,33], as we did in this study. A direct comparison between the different methods found ambient NO to have an effect on mixed expired but not on single breath measurement [16,34]. In the present study, there was no obvious early peak in NO at the beginning of exhalation while breathing high NO concentrations; merely higher values because of the increased NO in large airways (Figure 3). The high NO in large airways under such condition will inevitably lead to higher NO concentrations in a mixed exhaled gas sample. In contrast, NOet was unaffected by inhaled NO level as was NOplat.

Nitric oxide in gas phase and in hemoglobin free solution is relatively stable [35], and any nitric oxide released by cells into the air space in the lungs, be it small airways, alveoli or large airways, is likely to linger for a few seconds and certainly long enough for the duration of a breathing cycle. A fraction of the nitric oxide in the alveoli is lost by diffusion into the blood and binding to hemoglobin, but a considerable fraction remains in the lungs and is eliminated in the exhaled air [27,34]. Therefore NOet could possibly represent air from the alveolar region of the lungs. Indeed, there is considerable evidence that the source of exhaled NO is from the alveolar region and small airways; it is detected in isolated lungs [36,37], in tracheostomized animals [2] and in human with bronchoscopy [27]. Persson et al. [38] showed that injection of nitroglycerine intravenously in rabbits lead to an increase in exhaled NO, further suggesting that exhaled NO comes from the alveolar region. Bortland et al. [39] concluded that NO detected in the exhaled air in human is produced in the alveolar region. In isolated pig lungs, Cremona et al. [37] found increased exhaled NO after intravenous acetylcholine and decreased NO when blood was added to the perfusate suggesting that NO is produced in the alveolar region. Tsujino et al. [27] and Corradi et al. [34] also found that in healthy subjects the last portion of the breath (mostly alveolar gas) contained a significant level of NO, as much as the fraction in the large airways. Based on such evidence, it is reasonable to suggest that NOet represents alveolar NO and any changes in exhaled NO may reflect changes in NO diffusing into the blood. Indeed Hyde et al. [25] suggested that changes in NO in the exhaled gas represents changes in diffusing capacity rather than changes in NO production in the airways. Clini et al. [40] came to similar conclusions. Production of NO from lung tissue into the alveolar space is likely to increase during inflammatory conditions such as asthma, which can lead to increase NO in the exhaled air. However, it is also possible that diffusion of NO from the alveoli into the blood may have decreased in certain disease conditions such as interstitial lung disease, leading to more NO remaining in the alveolar space and hence to an increase in exhaled NO. During treatment the opposite happens. Therefore, changes in exhaled NO during inflammatory condition or during treatment may have to be explained partly in term of changes in diffusing capacity, or in ventilation/perfusion matching.

Chemiluminescence NO analyzers are the most accurate and rapid, but are not very portable and are relatively expensive. Less expensive and portable electrochemical analyzers are made by several companies, but have a low response time that would preclude using them for breath by breath analysis [41]. These portable analyzers were designed for a slow single breath maneuver, and are acceptable for offline mixed expired gas measurement. The results from such analyzer are thought to be poorly reproducible [18] most likely due to the poor reproducibility of the single slow deep breath maneuver. Some clinicians continue to question the suitability of NO measurement with such portable NO analyzers for clinical management [19,42]. This lack of confidence in the NO measurement may be partly due to the strict ATS guidelines which were improved in the updated version [14]. More consensus exist for the practice guidelines that were published to guide clinicians on interpretation and use of exhaled NO values [43]. These practice guidelines rely on having confidence in the measurement of exhaled NO and would be of greater clinical application only if we are able to measure exhaled NO more reliably. A better method is needed that is not so dependent on a strict methodology, requires no special effort and is easier to perform. Perhaps monitoring of NO, breath by breath, and using NOet is a better way to measure exhaled NO. A simple method such as using NOet as a measure of NO production in the lungs and airways, along with practice guidelines for interpretation of the results [43] would contribute significantly in improving confidence in using exhaled NO in routine clinical practice.

In conclusion, we believe that measuring end tidal NO to assess NO production from the lungs is simple and maybe more reliable than

the single breath maneuver that is highly dependent on flow and is more difficult to perform. Although the differences between NOet and NOplat values were insignificant in healthy subjects, such differences have to be examined in patients with asthma or lung inflammation.

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