

# Exhaled nitric oxide

## *An emerging marker of inflammation in respiratory diseases*

Syed S. Habib, MD, FCPS.

### ABSTRACT

الأوكسيد النتريكي المزفور الجزئي (FENO)، تم إدخاله مؤخراً كعلامة لقياس الالتهاب وإجهاد الأوكسدة في الرئة. تمت مراجعة الأدوار العديدة لأوكسيد النتريك (NO) في فسيولوجيا الأمراض التنفسية على نطاق واسع. هناك دليل متناقض بخصوص الوظيفة المضبوطة لأوكسيد النتريك (NO) في أمراض الرئة. يولد الإنزيم iNOS تجمعات عالية جداً لأوكسيد النتريك عندما يواجه الجسم ردّ تحريضي يجذب البلعمات الكبرى الذي يولد أوكسيد النتريك (NO)، لذلك يشارك أوكسيد النتريك دفاع المضيف ضد الكائنات الحية المعينة. مقاييس (FENO) مفيدة في أمراض الربو، أمراض الرئة الإنسدادية (COPD)، التليف الكيسي، وتوسع القصبات. التقنية المستعملة (FENO) عُبرت بشكل جيد جداً، يتطلب نفس كمية الوقت الذي يحتاجه جهاز قياس التنفس، ويمكن استخدامه في الأطفال. أضاف القياس (FENO) بعد آخر إلى تصميم التأثيرات التنفسية المضادة، لأنه يسمح لكشف الردود التحريضية في غياب الضعف الوظيفي. تزود هذه المراجعة بصيرة إلى طرق المقياس، العوامل الفيزيولوجية التي تؤثر على (FENO)، وتفسير النتائج والأمراض المتعلقة بتغيرات في مستويات (FENO).

Fractional exhaled nitric oxide (FENO) is a recently introduced non invasive marker to measure inflammation and oxidative stress in the lung. The numerous roles of NO in respiratory pathophysiology have been extensively reviewed. There is contradictory evidence regarding the exact function of NO in lung diseases. In pathological states, the enzyme inducible NO synthase generates extraordinarily high concentrations of NO when the body faces an inflammatory response by attracting macrophages that generate NO and hence NO participate in host defense against specific organisms. Fractional exhaled nitric oxide measurements have been useful in asthma, chronic obstructive pulmonary disease, cystic fibrosis, and bronchiectasis. The technique used to measure FENO is well standardized, requires the same amount of time that spirometry takes, and is feasible to be performed in young children. Measuring FENO

has added another dimension to the determination of adverse respiratory effects because it allows detection of inflammatory responses in the absence of functional impairments. This review provides an insight into measurement methods, physiological factors affecting FENO, interpretation of results and diseases related to changes in FENO levels. This will help physicians in diagnosing and monitoring their treatments for different respiratory diseases.

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*From the Department of Physiology, College of Medicine, King Saud University, Riyadh, Kingdom of Saudi Arabia.*

*Address correspondence and reprint request to: Dr. Syed S. Habib, Assistant Professor & Consultant, Department of Physiology (29), College of Medicine, PO Box 2925, King Saud University, Riyadh 11461, Kingdom of Saudi Arabia. Tel. +966 (1) 4671607. Fax. +966 (1) 4672567. E-mail: shahidhabib44@hotmail.com*

The story of endogenously produced nitric oxide (NO) began in 1980, when Furchgott and Zawadski<sup>1</sup> showed that the vascular endothelium produces a powerful vaso-dilating substance and named it endothelium-derived relaxing factor (EDRF). Seven years later, Palmer et al<sup>2</sup> and Ignarro et al<sup>3</sup> suggested that EDRF and NO were identical. Soon the fact was explored that such a simple molecule could exert so powerful effects. A rapidly increasing list of publications established NO as a vasodilator, bronchodilator, neurotransmitter, and an important component of the immune system.<sup>4</sup> Fractional exhaled nitric oxide (FENO) is an emerging inflammatory marker for respiratory pathologies with new and exciting data coming up. This will help physicians in diagnosing and monitoring their treatments for different respiratory diseases. This review will provide an insight into the measurement methods, interpretation, and diseases related to change in FENO levels.

*History of NO in exhaled air.* In 1991, Gustafsson et al<sup>5</sup> described the measurement of NO in the exhaled air of humans, rabbits, and guinea pigs leading to the

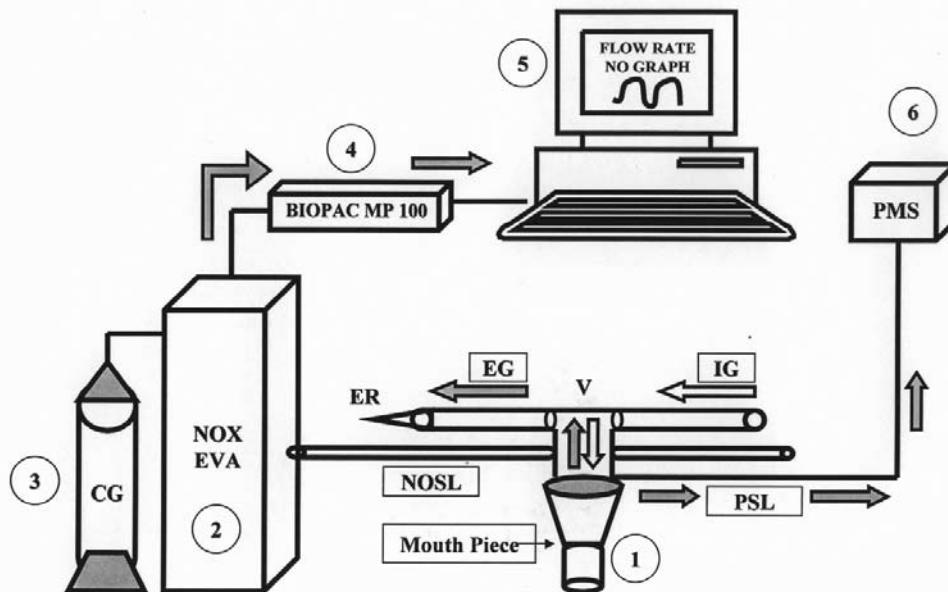
eventual development of commercial instruments for the real time measurement of FENO. The numerous roles of NO in respiratory pathophysiology have been extensively reviewed. Nitric oxide is an endogenous messenger with a diverse range of effects including non-adrenergic, non-cholinergic neurotransmission, vascular and non-vascular smooth muscle relaxation. There is contradictory evidence regarding the exact function of NO in lung disease. In pathological situations, NO is a pro-inflammatory mediator with immunomodulatory effects.<sup>6</sup>

**Synthesis of NO.** Nitric oxide is produced from the conversion of L-arginine to NO and citrulline by nitric oxide synthases (NOSs).<sup>7</sup> Nitric oxide is generated by 3 distinct isoforms of NOSs; neuronal NOS, inducible NOS, and endothelial NOS. Constitutive expression of NOS1 produces low levels of NO in healthy lungs. Inducible nitric oxide synthase 2A (NOS2A) is thought to be responsible for the increased levels of NO produced in inflammatory states in the lung, and is markedly upregulated by interferon- $\gamma$ , tumor necrosis factor- $\alpha$ , and interleukin-1 $\beta$ , and down-regulated by corticosteroids. The enzyme iNOS generates extraordinarily high concentrations of NO when the body mounts an inflammatory response by attracting macrophages, which generate NO and hence, NO participate in host defense against specific organisms.<sup>8</sup> In biologic specimens, NO is highly reactive and is rapidly converted into peroxynitrite via its reaction with superoxide.<sup>9</sup> Nitric oxide is produced by pulmonary vasculature and regulates vascular tone. Deficiency of NO production in the pulmonary vasculature results in pulmonary hypertension. Nitric oxide also avidly binds to hemoglobin, which acts as a sink and prevents systemic vasodilatation. In addition, NO diffuses across tissues to hollow organs such as the bronchus, where it remains stable in the gaseous phase.<sup>10</sup>

**The need for a quick and simple inflammatory marker.** Conventional measures of asthma severity have combined assessments of symptoms, amounts of  $\beta$ 2-agonist used to treat symptoms, and lung function. These measures do not assess airways inflammation, may not provide optimal assessment for guiding therapy and correlate poorly with eosinophilic inflammation on bronchial biopsies. Fractional exhaled nitric oxide is a quick and simple inflammatory marker to assess the impact of treatment changes on inflammation and thus to guide asthma therapy. Still, large long-term outcome trials are necessary to validate its usefulness. Routine measurement of FENO in our clinical settings still remains unclear, although current studies are encouraging that it runs in parallel to ongoing inflammation in a wide range of patients.<sup>11,12</sup>

**Measurement of fractional exhaled NO.** Fractional exhaled nitric oxide is measured non-invasively with a chemiluminescence NO gas analyzer after NO reacts with ozone to produce an electronically excited nitric dioxide molecule. Fractional exhaled nitric oxide levels can be measured within a few minutes online during slow exhalation or offline from samples collected in bags. The ATS recommends single-breath FENO measurements from the mouth, for online measurements, beginning expiration from total lung capacity (TLC). American Thoracic Society standardized procedures are used for FENO measurement. The instrument is calibrated before use over the range of 0-100 parts per billion (ppb) with dilutions of a known NO source, and care is taken that atmospheric levels do not exceed 30 ppb, as this is known to affect FENO.<sup>13,14</sup> In our laboratory, FENO measurements are performed by NOX EVA 4000 chemiluminescence analyzer (Seres, France) with a sensitivity of one ppb according to the present recommendations of the ATS. Using online visual monitoring, the subjects are asked to inhale from residual volume to TLC and then, subjects perform a slow expiratory vital capacity manoeuvre with a constant standardized expiratory flow rate of 0.05 L/sec ( $\pm$  10%) resulting in an expiration time of approximately 20 seconds, into a Teflon cylinder connected to 3 mm Teflon tubing, without the nose clipped. To exclude nasal NO contamination, a small expiratory resistance of 1-2 cm water is applied. This expiratory resistance is controlled by a special pressure management system. The subjects inspire from atmospheric air or ideally from NO free air and expire in a restricted-breath configuration set up. In our laboratory, the expiratory flow rate is measured by data acquisition system of BIOPAC MP-100 (BIOPAC Systems Inc, USA). Plateau levels of FENO against time are determined and expressed as ppb. Mean exhaled NO concentrations are determined between 5 and 15 seconds after start of the expiration. Three successive recordings are made at least one minutes apart, and the mean is taken as final result. Nitric oxide concentrations are calibrated 2-3 times per week using a standard NO calibration gas. The fractional exhaled nitric oxide measurement set up in our laboratory is expressed in detail in **Figure 1**.

**Principles to follow during exhaled No measurement.**<sup>13</sup> **Nitric oxide from airways and nasal passages.** As discussed earlier, FENO is produced by the pulmonary vasculature and then diffuses to the airways lumen<sup>10</sup> in both the upper and lower respiratory tract thus conditioning exhaled gas with NO.<sup>15,16</sup> There may be significant contribution from the oropharynx.<sup>17</sup> Although gastric NO levels are very high, this does not appear to contaminate exhaled NO, probably because of



**Figure 1** - Fractional exhaled nitric oxide (NO) measurement set up. White arrows indicate flow of inspired gas and grey arrows indicate flow of expired gas. 1. Restricted breathing apparatus, 2. NO analyzer (NOX EVA), 3. Calibration gas cylinder (CG), 4. BIOPAC data acquisition system (BIOPAC MP 100). 5. Computer set up for expiratory flow rate and NO graphs, 6. Pressure management system (PMS). IG - inspired gas, EG - expired gas, ER - expiratory resistance, V - valves, PSL - pressure sampling line, NOSL - nitric oxide sampling line.

closed upper and lower esophageal sphincters.<sup>18</sup> Nasal NO accumulates in high concentrations relative to the lower respiratory tract. Accordingly, techniques that aim to sample lower respiratory NO should prevent contamination of the sample with nasal NO with regulation of exhaled air pressure.<sup>19,20</sup> Recent guidelines prefer not to use nose clips to avoid nasal contamination of NO. It is also important to record environmental NO especially if it is high. Standardized techniques must prevent the contamination of biological samples with ambient NO. Therefore, it is mandatory to record room NO concentrations at the time of testing.<sup>13</sup>

**Expiratory flow rate dependence.** Fractional exhaled nitric oxide concentrations from the lower respiratory tract exhibit significant expiratory flow rate dependence,<sup>21</sup> including measurement of NO in human nasal airway.<sup>22,23</sup> This flow dependence is characteristic of a diffusion-based process for NO transfer from airway wall to lumen and can be simply explained by faster flows minimizing the transit time of alveolar gas in the airway, and thereby reducing the amount of NO transferred. The rate of NO output, however, is greater at higher flow rates analogous to respiratory heat loss.<sup>20</sup> In view of this flow dependency, the use of constant expiratory flow rates is emphasized in standardized techniques. Breath holding causes accumulation of NO in the nasal cavity, lower airway, and probably in the

oropharynx, which causes NO peaks in the exhalation profiles of NO versus time during measurement. For this reason, the use of breath hold is discouraged by ATS.<sup>24</sup> We control expiratory flow rate through the BIOPAC pressure management system.

**Physiological factors affecting FENO.** Age, gender, and body surface area. In adults, there is no consistent relationship between exhaled NO level and age, however in children, FENO increases with age. There is no clear relation between FENO and gender. Although men appear to have higher FENO levels than women, however, there are variable reports regarding age and gender.<sup>25-27</sup> Nitric oxide output, corrected for body surface area, is higher in children younger than 11 years. More work needs to be carried out in this area to draw strong conclusions regarding this aspect. The ATS recommends in any case, that height and weight should always be reported to allow calculations of NO output/body surface area.<sup>28</sup>

**Nutritional status and smoking.** It is preferable that FENO recordings are made between meals. Patients should refrain from eating and drinking before NO analysis. An increase in FENO has been found after the ingestion of nitrate or nitrate-containing foods, such as lettuce. It is possible that a mouthwash may reduce the effect of nitrate-containing foods.<sup>29</sup> Drinking of water and ingestion of caffeine may lead to transiently altered

FENO levels.<sup>30,31</sup> Later studies have reported that caffeine intake decreases exhaled NO.<sup>32</sup> Until more is known, it is prudent when possible to refrain from eating and drinking for one hour before exhaled NO measurement, and to question patients on recent food intake. Reduced levels of FENO have been demonstrated in both current and ex cigarette smokers.<sup>33</sup> Probable reasons may be lower total airway NO flux in ex-smokers and reduced airway and alveolar NO concentrations in current smokers. Despite the depressant effect of smoking, smokers with asthma still have a raised FENO. The ATS recommends that subjects should not smoke in the hour before measurements, and short- and long-term active and passive smoking history should be recorded.

**Diurnal variation.** It is yet not known whether measurements need to be standardized for time of day. However, where possible, it is advisable to perform serial NO measurements in the same period of the day and always record the time. Fractional exhaled nitric oxide levels are higher in nocturnal asthma, but there was no circadian rhythm in 2 studies,<sup>34,35</sup> while another report showed a circadian pattern.<sup>36</sup>

**Exercise, respiratory maneuvers, and other factors affecting FENO.** During exercise, FENO and nasal NO fall, whereas NO output increases, and this effect may last up to one hour.<sup>37</sup> Others reported that FENO remains stable after exercise.<sup>38</sup> It is recommended to avoid strenuous exercise for one hour before the measurement. Spirometric maneuvers have been shown to transiently reduce FENO levels; therefore, NO analysis should be performed before spirometry.<sup>39</sup> The same principal applies to other effort dependant respiratory maneuvers, unless these can be shown to have no effect on FENO. The FENO maneuver itself and body plethysmography does not appear to affect plateau exhaled NO levels.<sup>40</sup> Fractional exhaled nitric oxide levels may vary with the degree of airway obstruction or after bronchodilatation, perhaps due to a mechanical effect on NO output. Depending on the setting, it may be prudent to record the time of last bronchodilator administration and some measure of airway caliber, such as FEV1.<sup>41,42</sup>

**Fractional exhaled NO and respiratory diseases.** Patients with high FENO exhibit atopy with or without asthma.<sup>43,44</sup> Other conditions with high FENO are chronic obstructive pulmonary disease<sup>45,46</sup> and bronchiectasis.<sup>47,48</sup> Upper and lower respiratory tract viral infections increase levels of FENO in asthma. Therefore, FENO measurements should be deferred until recovery.<sup>49</sup> Low levels of FENO have been described in cystic fibrosis and pulmonary hypertension.<sup>50</sup> A large body of data is emerging regarding the behavior of FENO in various respiratory diseases, which are out of the scope of this review and need a separate discussion.

**Fractional exhaled NO as a marker of asthma diagnosis and severity.** There is no well-defined range of normal values for FENO in the general population. Different studies have shown variation in values. Steerenberg et al<sup>51</sup> showed that in atopic children, exhaled NO levels correlate with bronchial hyperresponsiveness. Heffler et al<sup>52</sup> reported that the sensitivity and specificity of FENO for detecting asthma, using 36 ppb as cut-off point, were 78% and 60% and the positive and negative predictive values were 54% and 82%. Fractional exhaled NO levels had a sensitivity of 49% and 19% and a specificity of 61 and 96% for detecting subjects with asthma at cutoff levels of 20 and 50 ppb respectively.<sup>53</sup> Olivieri et al<sup>54</sup> measured online levels of FENO in more than 200 nonsmoking adults at 50 mL/s and reported a range of 2.6-28.8 ppb in men and 1.6-21.5 ppb in women. Olin et al<sup>55</sup> showed a median value of 15.8 ppb in the range of 11.9-21.4 ppb in healthy subjects. Based on our unpublished data in healthy individuals of Saudi Arabia, mean levels are higher than other populations. Moreover, in asthmatics with ongoing inflammation, even use of steroids could not bring FENO levels to that of controls.

**Medications and FENO.** The potential effect of drugs on FENO cannot be excluded, and so all current medication and time administered should be recorded. There is conflicting evidence on the effect of short-acting bronchodilators on FENO levels as changes in airway caliber may affect FENO, although FENO does not significantly change after use of a long-acting  $\beta_2$  agonist.<sup>56-58</sup> Exhaled NO falls after treatment with inhaled or oral corticosteroids,<sup>59,60</sup> inhaled NO synthase inhibitors,<sup>61</sup> and leukotriene-axis modifiers<sup>62,63</sup> in subjects with asthma. Nitric oxide donor drugs,<sup>64</sup> and oral, inhaled, and intravenous L-arginine<sup>65</sup> increase FENO and nasal FENO.<sup>66</sup> Even if a certain medication does not affect NO production, it might affect the apparent level of NO through other mechanisms, such as changes in airway caliber.

**Future prospects of FENO.** Fractional exhaled NO measurements offer a step forward in the assessment of airways disease. As an "inflammatory marker," FENO provides clinician with the status of airway inflammation, thus, complementing conventional physiological testing. Fractional exhaled NO measurements are easy to perform, reproducible, and technically less demanding than other traditional tests of pulmonary pathology. Fractional exhaled nitric oxide measurement is emerging as complementary conventional diagnostic and assessment tool for inflammatory lung diseases. The noninvasive and simplicity of its measurement technique makes it a useful guide in many respiratory pathologies. Still, there is need for more work before we can interpret the results with complete confidence.

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