Advances in Pulmonary Laboratory Testing*

Bruce D. Johnson, PhD; Kenneth C. Beck, PhD; R. Jorge Zeballos, MD; and Idelle M. Weisman, MD, FCCP

This review examines emerging technologies that are of potential use in the routine clinical pulmonary laboratory. These technologies include the following: the measurement of exercise tidal flow-volume (FV) loops plotted within the maximal FV envelope for assessment of ventilatory constraint during exercise; the use of negative expiratory pressures to assess expiratory flow limitation in various populations and under various conditions; the potential use of expired nitric oxide for assessing airway inflammation; and the use of forced oscillation for assessment of airway resistance. These methodologies have been used extensively in the research setting and are gaining increasing popularity and clinical application due to the availability of commercially available, simplified, and automated systems. An overview of each technique, its potential advantages and limitations will be discussed, along with suggestions for further investigation that is considered necessary prior to extensive clinical use. (CHEST 1999; 116:1377–1387)

Key words: flow-volume; forced oscillation; negative expiratory pressure; nitric oxide

Abbreviations: EELV = end-expiratory lung volume; EILV = end-inspiratory lung volume; extFVL = tidal flow-volume loop measurement during exercise; FO = forced oscillation; FV = flow-volume; IC = inspiratory capacity; HRV = inspiratory reserve volume; MFVL = maximal flow-volume envelope; MVV = maximum voluntary ventilation; NEP = negative expiratory pressure; NO = nitric oxide; NOS = nitric oxide synthase; Raw = airway resistance; Rti = resistance to movement of lung tissue; TLC = total lung capacity; V˙e = minute ventilation; Vt = tidal volume; WOB = work of breathing

Many advances in the pulmonary laboratory have occurred over the last 2 decades. The majority of these advances involve automation of routine pulmonary function measurements, refinement of the diffusing capacity test, innovation of fast-response analyzers to obtain “real time” cardiopulmonary exercise data, and progression into the standard use of the body plethysmograph for assessment of lung volumes and airway resistance. Although there are many techniques that are applicable primarily in the research setting, there are several emerging methods that may offer unique clinical insight or may offer advantages over more “traditional” techniques. The following sections offer a brief review of several methods that are gaining popularity or, through advances in technology, are becoming available in the clinical setting. These include the following: (1) the use of the tidal flow-volume (FV) loop measurement during exercise (extFVL) to help distinguish the degree of ventilatory limitation; (2) the use of the negative expiratory pressure (NEP) technique to detect expiratory flow limitation; (3) the use of expired nitric oxide (NO) in the assessment of airway inflammation; and (4) the forced oscillation (FO) technique to assess airway resistance.

Assessment of Ventilatory Limitation Using the extFVL

There has been a growing trend in both research and clinical laboratories to find alternative ways to evaluate ventilatory limitation during exercise.1–5

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This stems in part from the realization that patients may discontinue exercise due to ventilatory constraints and dyspnea prior to the achievement of classic indexes associated with ventilatory limitation (ie, minute ventilation [Ve] that reaches the maximum voluntary ventilation [MVV] or a rise in arterial CO_2) and that Ve limitation is not an “all or none” phenomenon. Thus, investigators have used techniques such as breathing helium-oxygen mixtures (to increase the maximal FV envelope [MFVL]), inspired CO_2, and dead space loading (to stimulate ventilation) to assess whether Ve is truly constrained. Another approach that has gained popularity includes the measurement of the extFVL and its plotting within the MFVL. This latter technique provides a good visual index of the degree of Ve constraint, allows a more detailed approach to defining Ve limitation (relative to the Ve/MVV relationship), and has gained popularity due to the ease of measurement using many of the commercially available metabolic carts. Figure 1 shows an example of the rest and peak exercise FV responses in a healthy, average fit adult plotted within the MFVL. Key features of the Ve response in the healthy adult are shown in Table 1. In this particular example, at peak exercise, there is only minimal encroachment on the MFVL, which implies that there is little constraint to breathing.

Methodology

Commercially available metabolic carts use various flow sensing devices to measure the MFVL at rest and FV responses during exercise, typically in conjunction with standard gas-exchange measurements. Two or three MFVLs are produced at rest prior to exercise in addition to several maximal inspiratory maneuvers from functional residual capacity. Exercise tidal FV responses (typically two to five) are obtained toward the end of each work intensity, followed by repeat inspiratory capacity (IC) maneuvers. The IC measurements are used to place the tidal loops within the MFVL by aligning the maximal inspiratory volume points at total lung capacity (TLC). The change in IC, therefore, represents changes in end-expiratory lung volume (EELV), assuming that TLC does not change. Since bronchodilation or constriction may occur with exercise, MFVLs may also be obtained either during or immediately after exercise. Previous studies have taken single representative tidal exercise breaths or a mean of two or more breaths to plot within the largest MFVL obtained.

Information Gained From the extFVL

Varied information has been gleaned from the extFVL plotted within the MFVL, from simple visual information to actual quantification of various indexes of Ve constraint. To date, no consensus exists on which variables will prove to be the most useful clinically or how best these may be quantified. The most commonly reported variables are shown in Table 2. Other investigators have used various additional indexes, such as tidal volume (VT) relative to IC or vital capacity in conjunction with breathing frequency to help describe the degree of Ve constraint.

Clinical Application

The extFVL has been examined in a number of populations. Varied information has been obtained,
The ad-

Dynamic rise in EELV Decrease in IC (EELV = IC) May cause dynamic compression of airways, reflex inhibition of IC, increases WOB and cost of breathing

Table 1—Key Features of the $\dot{V}e$ Response to Exercise in a Healthy Adult*

<table>
<thead>
<tr>
<th>Constraint</th>
<th>How Indices Are Assessed</th>
<th>Proposed Role in $\dot{Ve}$ Constraint</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decrease in lung volume at the end of a normal expiration (↓ EELV) through recruitment of expiratory muscles</td>
<td>Optimizes inspiratory muscle length</td>
<td></td>
</tr>
<tr>
<td>Vt increases through equal encroachment on the IRV and ERV</td>
<td>Vt is kept on the linear portion of the pressure-volume relationship of the lung and chest wall</td>
<td></td>
</tr>
<tr>
<td>Expiratory flow rates remain within the maximum available flow rates (particularly at the higher lung volumes) Avoidance of high EILVs Inspiratory flow rates are well within the maximal available flows</td>
<td>Minimal expiratory flow limitation Reduces the elastic load Inspiratory flow reserve</td>
<td></td>
</tr>
</tbody>
</table>

*ERV = expiratory reserve volume.

including the specific source and degree of $\dot{Ve}$ constraint, possible mechanisms of associated dyspnea, as well as effectiveness of treatment (e.g., surgery or bronchodilator therapy). The advantages over a traditional assessment of $\dot{Ve}$ reserve ($\dot{Ve}$/MVV ratio) includes more specific information on the mechanisms of $\dot{Ve}$ constraint and more formal quantification of the degree of constraint. Although not yet generalizable to the larger population, smaller studies have suggested that in patients with asthma and mild-to-moderate emphysema, and in healthy older subjects (subjects with mild-to-moderate obstructive airway changes) EELV will dynamically increase during exercise to varying degrees as expiratory flow limitation develops to take advantage of the flow available at the higher lung volumes. The increase in EELV in turn causes greater encroachment on the IRV (the EILV/TLC ratio, which increases elastic load) and potential encroachment by the inspiratory tidal loop on the inspiratory FV curve. In patients with more significant obstructive disease, the magnitude of the rise in EELV, as assessed with serial IC maneuvers, correlates more strongly with exercise endurance and dyspnea than do traditional measurements of FEV$_1$. Thus, in some populations, the change in the EELV at rest or during exercise may represent the most sensitive marker of ventilatory constraint. Patients with interstitial lung disease do not appear to demonstrate significant increases in EELV during exercise, while patients with chronic heart failure and subjects with significant obesity may actually breathe with reduced EELVs despite significant expiratory flow limitation and a normal $\dot{Ve}$/MVV relationship.

Limitations to the widespread use of the extFVL in the clinical setting include difficulty in defining the true MFVL, effort dependence of the IC maneuvers, and constancy of TLC. Small errors in either of these measurements or assumptions could lead to erroneous conclusions. Partial MFVLs or maneuvers of MFVLs at different levels may be necessary to better define the maximal expiratory flow boundary in some patient populations. Additional studies are necessary to determine the following: (1) the optimal MFVL for comparison to the extFVL; (2) the ability of patients to perform multiple IC maneuvers; (3) which indexes are most clinically useful in describing the degree of $\dot{Ve}$ constraint (e.g., the percentage of $\dot{Ve}$ flow limited, increase in EELV, or increase in EILV/TLC ratio); (4) the relationship of various indexes of constraint to measurements of dyspnea and exercise intolerance; and (5) the degree of constraint that begins to influence exercise tolerance (e.g., exercise capacity, perception of dyspnea, and cardiovascular function).

The use of the extFVL would be helpful in many

Table 2—Indices of Ventilatory Constraint Using extFVLs Plotted Within the Maximal FV Envelope

<table>
<thead>
<tr>
<th>Indices of $\dot{Ve}$ Constraint</th>
<th>How Indices Are Assessed</th>
<th>Proposed Role in $\dot{Ve}$ Constraint</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expiratory flow limitation</td>
<td>% Vt meeting or exceeding the expiratory boundary of the MFVL</td>
<td>May cause dynamic compression of airways, reflex inhibition of Vt, increases WOB and cost of breathing</td>
</tr>
<tr>
<td>Inspiratory flow reserve</td>
<td>Peak inspiratory flow during exercise relative to the maximal flow available</td>
<td>High WOB and cost of breathing, respiratory muscles working in a fatiguing range</td>
</tr>
<tr>
<td>Elastic load</td>
<td>EILV/TLC ratio</td>
<td>Increased WOB and cost of breathing, inspiratory muscles more easily fatigued</td>
</tr>
<tr>
<td>Dynamic rise in EELV</td>
<td>Decrease in IC (EELV = TLC – IC)</td>
<td>Reduces inspiratory muscle length, increases elastic load, WOB and cost of breathing</td>
</tr>
<tr>
<td>$\dot{Ve}$ capacity</td>
<td>Calculated $\dot{Ve}$ based on the extFVL, EELV and MFVL</td>
<td>Overall assessment of breathing reserve, $\dot{Ve}$/Ve capacity</td>
</tr>
</tbody>
</table>

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patient groups in which dyspnea and exercise intolerance were noted and mechanical constraints to breathing are a possibility. In patients with normal lung function or with severe airway changes (eg, $FEV_1 < 30$ to $40\%$), such an assessment is unlikely to yield significant additional insight, however, in patients with more moderate obstructive changes or to assess the effectiveness of treatment, further evaluation using the extFVL may be valuable. Figure 2 shows an example of the rest and extFVLs plotted within the MFVL in a physically active patient with moderate COPD. Interestingly, this patient had a normal exercise capacity for his age, however, he clearly could not increase $VE$ further due to the degree of constraint (ie, increased EELV and significant expiratory flow limitation.)

It should also be emphasized that the degree of $VE$ constraint is not only dependent on the area circumscribed by the MFVL and the breathing strategy, but also on the $VE$ demand. Thus, a highly fit healthy adult may encroach to a similar degree on the MFVL as a significantly deconditioned patient with some obstructive airway disease due to a significantly higher $VE$.7,10

**Assessment of Expiratory Flow Limitation Using Negative Expiratory Pressures**

Expiratory flow limitation promotes a dynamic increase in EELV with a concomitant increase in inspiratory work, impairment of inspiratory muscle function, and adverse effects on hemodynamics.19 These changes, combined with the dynamic compression of airways, likely contribute to dyspnea in patients with obstructive airway disease.11 The NEP technique evolved out of a need for an accurate assessment of expiratory flow limitation.20 It was originally used to assess expiratory flow limitation during mechanical ventilation, however, it has more recently been applied to various patient populations during breathing at rest and during exercise.21–25

When assessing flow limitation using the tidal breath method relative to the expiratory boundary of the MFVL, it remains controversial how to best define the maximal expiratory flows for comparison to the tidal FV loop. This is due to several factors, which have been shown to have varying influences on the size of the MFVL, as summarized in Table 3.9,13,26–28

In addition, the placement of the tidal FV loop is dependent on the accuracy of the IC maneuvers. To illustrate these potential problems in defining maximal expiratory flows, some studies have observed that tidal flows exceed the MFVL during exercise.9,10 This suggests, therefore, that the point where the tidal flows meet the expiratory boundary of the MFVL represents “impending” flow limitation (the point at which pleural pressure begins to increase out of proportion to flow) rather than “true” flow limitation (the point at which flow plateaus relative to pleural pressure) in some patient populations.7

In healthy subjects, the influences of dynamic compression, lung volume, and flow history on the expiratory boundary of the MFVL are likely to be small, however, they can become more substantial in patients with obstructive airway changes.11,26,27 Although these changes can generally be accounted for by varying expiratory effort when performing the MFVL maneuvers, by performing partial MFVL maneuvers, or by repeating maneuvers if airway caliber is suspected to change (eg, during or after

**Table 3—Factors Influencing the Maximal Expiratory Flows During a Forced Maneuver**

<table>
<thead>
<tr>
<th>Suggested Influence on the MFVL</th>
<th>Suggested Mechanism of Altering Maximal Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excessive expiratory pleural pressure generation</td>
<td>Causes dynamic compression of airways</td>
</tr>
<tr>
<td>Lung volume history prior to performing the MFVL</td>
<td>Alters viscoelastic forces, airway resistance</td>
</tr>
<tr>
<td>Speed of inspiration preceding the MFVL maneuver</td>
<td>Affecting elastic recoil</td>
</tr>
<tr>
<td>Exercise-induced changes in airway function</td>
<td>Bronchoconstriction or bronchodilatation</td>
</tr>
</tbody>
</table>

Figure 2. Example of the FV response to exercise in a patient with moderate obstructive airway changes. EELV increases with only moderate exercise due to expiratory flow limitation. By peak exercise the patient experiences both expiratory and inspiratory flow constraint and approaches a high EILV/TLC ratio. Clearly, little room exists to increase flow or volume relative to the profile of healthy adult shown in Figure 1.

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exercise), these technical concerns can, for the most part, be eliminated using the NEP technique to assess expiratory flow limitation.

**Methodology**

The NEP technique was introduced for detecting expiratory flow limitation, which does not require the performance of forced expiratory efforts on the part of the patient, use of a body plethysmograph to correct for gas compression, or the performance of IC maneuvers.\(^{20}\) It consists of applying a negative pressure at the mouth during a tidal expiration and comparing the ensuing expiratory flow curve with that of the previous control expiration.\(^{21}\) Typically, subjects are attached to a mouthpiece connected in series to a pneumotachograph and a T-tube. One side of the T-tube is open to the atmosphere, while the other side is equipped with a pneumatic valve, which allows for the subject to be rapidly switched to a negative-pressure source (eg, a vacuum). The negative-pressure source typically has been set at −3 to −10 cm H\(_2\)O in various populations.\(^{19,24,29}\) NEP is generally applied at the initiation of expiration and is maintained throughout most of expiration for comparison to non-NEP control breaths. Figure 3 shows an example of the NEP technique applied in a subject who is not flow limited (ie, who has an increase in flow during NEP compared with a control breath) during spontaneous breathing and in a patient who is flow limited (ie, who has no increase in flow with NEP compared with a control breath).\(^{21}\)

Commercial systems are becoming available for interface with existing metabolic carts.

The application of NEP during expiration increases the pressure gradient between the alveoli and the airway opening.\(^{20}\) The induction (initial onset) of NEP results in an artificial increase in expiratory flow in both flow-limited and non-flow-limited patients. This is thought to be due to a reduction in volume from the more compliant oral and neck structures as well as from the larger intrathoracic airways.\(^{21,30}\) Generally, use of NEP also will result in an augmented expiratory volume, particularly if flow limitation does not exist. In flow-limited subjects, the use of NEP will augment dynamic compression, however, this is thought to occur downstream from the flow-limiting segments and, thus, typically should not influence the available maximal expiratory flow in these patients.\(^{21}\)

**Clinical Application**

The NEP method has been applied to assess whether expiratory flow limitation exists during spontaneous breathing in patients with COPD,\(^{20}\) before and after single lung transplantation,\(^{19}\) in patients with stable asthma,\(^{23}\) and in patients with restrictive respiratory disorders.\(^{24}\) It has also been successfully applied during mechanical ventilation\(^{20}\) and in infants with cystic fibrosis,\(^{22}\) as well as during exercise in COPD patients.\(^{25}\) The degree of expiratory flow limitation obtained with NEP also has been shown to be more highly correlated with dyspnea than other indexes of pulmonary function (eg, FVC and FEV\(_1\)) in COPD patients.\(^{26}\)

Although an effect similar to NEP could be obtained by asking subjects to simply augment expiration slightly during a transient breath, the NEP technique has the advantage of detecting expiratory

![Figure 3](http://journal.publications.chestnet.org/pdfaccess.ashx?url=data/journals/chest/21933/ on 06/24/2017)
flow limitation in patient groups that cannot easily respond to instruction. In addition, it is possible that breathing mechanics immediately preceding the expiration may be altered slightly when individuals are asked to prepare to augment a subsequent expiratory effort.

In subjects spontaneously breathing at rest, the application of NEP at the onset of expiration has sometimes resulted in a drop in flow below the flow rate generated during the preceding tidal expiration, presumably due to reflexive partial or total upper airway narrowing. A subsequent study, however, suggested that reflex-mediated changes in upper airway diameter (genioglossus) tended to occur near the end of expiration rather than at the initiation, except in a few subjects. Another potential limitation to the NEP technique is the inability to quantify the degree of expiratory flow limitation. Early expiration cannot be easily assessed due to the spike artifact caused from NEP. However, this artifact is quite brief, and, typically, flow limitation occurs predominantly over the later portion of expiration. Thus, in most subjects the percentage of the tidal breath that is limited can be quantified, except perhaps in subjects in whom expiratory time is significantly reduced. The NEP technique alone does not provide insight on other markers of VE constraint, such as the change in EELV or an index of elastic load. It also does not provide insight into inspiratory flow constraints, which may occur in patients with muscle weakness or fatigue or in those with significant hyperinflation. Unless the NEP technique is performed with the assessment of the tidal FV loops, flow limitation is defined as “all or none” rather than in terms of the graded degrees of limitation defined with the tidal loops. An all-or-none assessment of expiratory flow limitation tells little about the degree of VE constraint, especially since subjects may augment VE despite flow limitation by dynamic hyperinflation or further encroachment on the IRV. Thus, for the assessment of ventilatory constraint, further studies are necessary to determine which indexes (e.g., the presence of flow limitation, degree of flow limitation, change in IC, or some other index) will represent the most sensitive markers of VE limitation. A combination of the NEP technique along with traditional estimates of breathing reserve and use of the extFVFL may provide the greatest amount of information on VE constraints imposed by the lung and chest wall.

Although the use of NEP and the plotting of the tidal FV loops are fairly easily applied techniques that advance our understanding of VE limitation in various populations without the need for more invasive studies (e.g., esophageal and gastric balloons), other factors that may contribute significantly to exercise limitation involving the pulmonary system clearly cannot be negated (i.e., the work and cost of breathing, pulmonary hemodynamics, pulmonary influences on cardiac function, varying perceptions of dyspnea, and gas-exchange abnormalities).

Exhaled NO Measurement

NO is a highly reactive molecule formed by the enzyme NO synthase (NOS) from the precursor amino acid l-arginine. It was first described as an endogenous endothelium-derived nitrovasodilator that acts by directly activating guanylate cyclase, leading to increased cytosolic guanylate monophosphate by forming a reversible adduct with the heme moiety of the enzyme. Similarly, it may be readily inactivated by hemoglobin or cytochromes; thus, NO released into the blood stream is rapidly scavenged. It is a colorless gas that also can be carried in solution in plasma or in lipid membranes. In gaseous form, NO reacts directly with oxygen to form N2O, but in the absence of oxygen it can be quite stable. Its half-life in biological media is also quite short.

The roles of NO in physiologic processes are still being elucidated. It acts as a neurotransmitter in many tissues, including the nonadrenergic noncholinergic nervous system that innervates the pulmonary airway. There are at least four isoforms of the enzyme NOS: three constitutive forms and one inducible form. The constitutive forms require calcium for activation and produce a steady background low level of NO, whereas the inducible form of NOS is not calcium dependent and plays a role in adaptive processes such as inflammation. In the lung, the constitutive enzymes are found in endothelial cells, epithelial cells, smooth muscle cells, and inflammatory cells.

Given the presence of NOS in many of the cell types of the lung, it should be no surprise that NO can be measured in exhaled air. Since the first description of NO in exhaled air, much work has been done to characterize the source of NO and to correlate exhaled NO with various disease states. It is important to distinguish exhaled concentration of NO (usually expressed in parts per billion) from NO output (usually expressed in nanograms per minute). Moreover, mixed expired NO must be distinguished from instantaneous NO measurements. Exhaled NO can thus be documented in at least four ways: the NO output, the mixed expired NO, and either peak NO or end-expiratory NO. NO output from the lungs reflects a balance of pulmonary production less the diffusion of NO across the capillaries into the blood. The European Respiratory Society has issued guidelines for measurement of expired NO in an
effort to standardize the technique and to optimize detection of pulmonary rather than nasal NO.

Where is the exhaled NO coming from? Exhaled NO was documented in isolated lung perfused with blood-free medium, indicating that the NO was not derived from the circulation. The nose is a significant source of NO that can be admixed in gas sampled at the mouth.34 The specific cell that produces the measured NO has not been clearly defined, though most investigators assume the source of NO is either the epithelial cell, the endothelial cell, or nonadrenergic, noncholinergic neurons, although mast cells, macrophages, and eosinophils could also be significant sources.

There have been numerous studies correlating exhaled NO to various disease states (Table 4). Because the technical details of isolating lower from upper airway contributions have progressed simultaneously with correlations against disease states, much of this work bears repeating with standard methods.33

The relatively specific increase in exhaled NO in untreated or steroid-resistant asthma should be evident in Table 4. The role of NO in the pathogenesis of asthma is not fully determined. Although NO is a mild bronchodilator when inhaled from exogenous sources, increasing levels of exhaled NO by using inhaled L-arginine (the precursor for NO) causes acute bronchoconstriction,35 which is possibly mediated by an increase in vascular permeability that is mediated by high local concentrations of the endogenously released NO. NO also enhances eosinophil survival in vitro,36 which is consistent with the correlation between NO and eosinophil levels and suggests a role for NO in maintaining airway inflammation.

### Laboratory Requirements

The cost of NO analyzers is currently quite high, in the range of $20,000 to $40,000. Most analyzers were designed for monitoring the delivery of NO, so they are not designed for documentation of exhaled NO. The ideal instrument for measuring exhaled NO would be easy to calibrate and maintain, and it would be integrated into a system with a mouth pressure transducer and a flowmeter to monitor exhaled volume, and possibly a flow restrictor to reduce nasal contamination and to control for the effects of exhaled flow on the level of NO. Computer software would allow the display of concentration vs time graphs and the calculation of various parameters from the tests. Such instruments are just starting to become available. Thus, to set up exhaled NO measurement for clinical or research applications, one may need a high level of expertise in setting up instrumentation, and in acquiring and analyzing data.

In conclusion, the measurement of exhaled NO is a potentially useful test that could be performed in the setting of routine pulmonary function testing to document the state of airway inflammation in patients with asthma. The test appears to be relatively specific for inflammation related to airway eosinophilia and could prove to be a useful indicator of the effectiveness of antiinflammatory therapy in asthma. However, standard methods must be used to avoid contamination by the nose and to standardize the effects of expiratory flow on the measurement. Furthermore, large population studies using standard methods would be useful to determine precise population 95% confidence intervals for the measurement.

**Table 4—Expired NO in Lung Diseases**

<table>
<thead>
<tr>
<th>Disease or Condition</th>
<th>Comment</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated NO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asthma</td>
<td>Expired NO reduced by NOS inhibitors, indicating NOS as the source,40 High correlation with eosinophilia in induced sputum,47,48</td>
<td>Dupont et al40, Kharitonov et al40, Persson et al41, Stirling et al52, Wang et al53</td>
</tr>
<tr>
<td>Steroid-resistant TB</td>
<td>Exhaled NO remains elevated</td>
<td></td>
</tr>
<tr>
<td>TB</td>
<td>NO may be derived from alveolar macrophages, as increased iNOS was demonstrated on macrophages from patients with active TB.</td>
<td></td>
</tr>
<tr>
<td>Reduced NO</td>
<td>Low NO may be explained by reduced expression of iNOS in CF airway epithelial cells, hindering local defense mechanism, particularly in the killing of <em>Pseudomonas aeruginosa</em></td>
<td>Lundberg et al54</td>
</tr>
<tr>
<td>CF</td>
<td></td>
<td>Mazzi et al55</td>
</tr>
<tr>
<td>COPD</td>
<td></td>
<td>Brett and Evans56</td>
</tr>
<tr>
<td>ARDS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asthma, steroid treated</td>
<td>Correlation with methacholine PD20 is poor in treated asthmatics</td>
<td>Horvath et al57</td>
</tr>
</tbody>
</table>

*TB = tuberculosis; CF = cystic fibrosis; iNOS = inducible NOS; PD20 = provocative dose causing a 20% fall in FEV1.*

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Resistance to the movement of gas is calculated from the ratio of pressure change to flow. Resistance is of interest from both a basic science and a clinical perspective because it is an important determinant of the work of breathing (WOB). Total resistance of the respiratory system is a lumped sum of resistance to movement of lung tissue (Rti) and airway resistance (Raw) to the flow of gas. The sum of Rti and Raw for the lung is often called lung or pulmonary resistance. The measurement of Raw would seem to be the most clinically useful parameter as it reflects the state of the airways, though Rti is increasingly recognized as contributing significantly to the WOB at normal breathing frequencies. However, separating these two components of resistance requires very technically demanding or highly invasive techniques that are available only in animal laboratories.

To determine Raw, one needs to know instantaneous values for flow and the pressure that drives flow through the airways at multiple points in the breathing cycle. Body plethysmographs report the average Raw of the entire airway tree from mouth to the “average” alveolus. Plethysmographs are large and expensive, and some patients find it difficult to perform the panting maneuver required for procedure. Thus, a technique to measure resistance that would involve smaller, cheaper equipment and that would be easy to use for both patients and technical personnel potentially would be useful in a clinical setting.

The FO technique has the potential to fill this

<table>
<thead>
<tr>
<th>Problem</th>
<th>Comments</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rrs</td>
<td>Attempts to model system may help derive meaningful parameters from measurements (eg, large airway Raw, peripheral airway Raw, chest wall)</td>
<td>Peslin and Fredberg¹⁰</td>
</tr>
<tr>
<td>Effects of inhomogeneous Rrs and regional lung compliance</td>
<td>Complicates interpretation of patterns of Rrs vs frequency; probing with lower frequency than commercial devices are capable of may help</td>
<td>Lutchen et al⁵⁸</td>
</tr>
<tr>
<td>Glottic aperture may narrow during quiet breathing (expiratory braking)</td>
<td>Resistance values dominated by upper airway rather than lung airways</td>
<td>Stanescu et al⁵⁹</td>
</tr>
<tr>
<td>Peripheral airways may be “silent zone”</td>
<td>Changes in peripheral airways may be best early marker of disease; these changes may be undetectable by commercial units</td>
<td>MacKlein et al⁶⁰</td>
</tr>
<tr>
<td>Low reproducibility and wide range of normal values</td>
<td>Large changes required to attain clinical significance; possible reducing sensitivity compared to spirometry</td>
<td>Lebecque and Stanescu⁴³, Cuijpers et al⁶¹, Timonen et al⁶²</td>
</tr>
</tbody>
</table>

*Rrs = total respiratory system resistance.

### FO Resistance Measurement

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### Table 5—Problems and Drawbacks of the FO Technique

<table>
<thead>
<tr>
<th>Advantage</th>
<th>Comment</th>
<th>Study</th>
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<tbody>
<tr>
<td>Quiet breathing maneuver is simple to perform</td>
<td>Some patients may still be unable to tolerate mouthpiece, or have trouble keeping tongue out of the way</td>
<td>Ducharme and Davis⁶³</td>
</tr>
<tr>
<td>Separate values for large airway and peripheral airway resistance</td>
<td>These data come from models, which may not be standardized among implementations, and must be proven with larger studies</td>
<td>Lutchen et al⁵⁸</td>
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<td>No deep inspiration required; avoids airway collapse and flow limitation</td>
<td>Reversibility of resistance by deep inspiration may be defect in asthma: testing with no deep inspirations may “mask” true asthma</td>
<td>Skloot et al⁶²</td>
</tr>
<tr>
<td>Useful in challenge studies</td>
<td>Normal values must be determined in large population studies; adequate comparison must include separate sessions using spirometry and FO (see comments on deep inspiration)</td>
<td>Bohadana et al⁶⁰, Schmekel and Smith⁴¹</td>
</tr>
<tr>
<td>May be useful in monitoring situations (eg, critical care or sleep)</td>
<td>May be useful in monitoring situations (eg, critical care or sleep)</td>
<td>Badia et al⁸⁴</td>
</tr>
<tr>
<td>May be used in younger patients compared to spirometry</td>
<td>A higher proportion of 2- to 6-yr olds could complete FO tests compared to spirometry</td>
<td>Ducharme and Davis⁶³</td>
</tr>
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</table>
need. With FO, resistance of the pulmonary system (ie, airways, lung, and chest wall) is determined by imposing known variations in flow at the mouth and by measuring the resultant pressure changes. Early studies used pure sine waves at fixed frequency, though later studies probed with multiple frequencies to find the resonant frequency (the point at which compliance effects and inertial effects cancel, giving pure flow resistance). In the early 1970s, techniques were developed to probe a frequency range of interest with a more complex forcing function, using either “white noise” (a sound wave with a uniform representation of frequencies) or a series of brief impulses in flow at the mouth. With both innovations, the patient was required only to breathe quietly while the imposed forcing function was applied and measurements were made over a period of about 1 min. Because the frequency of the imposed oscillations was much higher than that of the patients’ breathing, the effects of the normal breathing cycle could be subtracted out. White noise and flow impulses both contain a range of frequencies, and mathematical techniques were used to separate out the individual frequency components to determine frequency dependence of the resistance and compliance. The equipment, mathematical analysis, and data-handling techniques have been technically very demanding for both these techniques. Recently, several manufacturers have made impressive efforts to miniaturize the equipment and to automate the data analysis and data handling to improve the user-friendliness of the FO technique. The equipment involved is considerably smaller than the body plethysmograph, and equipment setup and calibration have been automated so that technical personnel with little training can use the equipment. However, the investigator must be aware of what resistance value these devices are reporting and of both the physiologic and technical limitations of the technique (Table 5).

The fact that FO measures the total pulmonary system resistance, and that peripheral Raw, obtainable only through conceptual and mathematical modeling, may be masked by effects in larger airways may seriously limit the usefulness of the method. Results from the technique can be reported in several ways. Total resistance values at particular frequencies may be the best parameters as they are independent of models, which may vary among different implementations. A frequency of 8 Hz is a good candidate because it is near the resonant frequency of the respiratory system. The resonant frequency of the system or the ratio of the resistance at low frequency to that at high frequency may also be useful, but studies showing their sensitivity to disease are just starting to be produced. An alternative is to report the resistance obtained from fitting conceptual or mathematical models to the data. However, the investigator must realize that resistance at any given frequency is total respiratory system resistance and that the model-fitting procedures remain to be proven in clinical practice with large population studies.

There are several potential advantages of the FO technique over both spirometry and plethysmography (Table 6). The technique has potential advantages for challenge testing, as multiple tests at many dose levels will be easier to tolerate for the patient. However, the investigator must realize that the test does not require a deep inspiration. There is increasing evidence that a major defect in asthma is the reduced or paradoxical response to deep inspirations or sighs. Challenge testing without deep inspiration reduces the difference between patients with asthma and healthy subjects. A thorough comparison study must include dose-response determination in both healthy subjects and patients with asthma using FO and spirometry on separate days (with and without deep inspirations).

**Laboratory Requirements**

The commercially available instruments are much smaller than their traditional research counterparts and are much easier to use and maintain. The cost of the instruments is in the range of $12,000 to $20,000. Laboratory space requirements are minimal: the instrument is usually mounted on an adjustable arm attached to a small table or cart. Technical personnel familiar with spirometry or other pulmonary function tests will not have difficulty learning the techniques to use and calibrate the instruments. However, it should be stressed that the devices cannot be considered maintenance free. Like spirometers or body plethysmographs, they contain flowmeters and pressure transducers the accuracy of which is essential to obtaining accurate data.

Will these devices replace spirometry or plethysmography in the routine testing laboratory? Probably not, in the near future. Several studies have shown results of FO testing to be no more sensitive than spirometry in detecting disease or in correlating with symptoms. Because standard reporting procedures are not firmly established, and because there are only a few large population studies showing the range of normal and the patterns of abnormality with disease, the interpretation of the data should be performed very conservatively.

In conclusion, the determination of pulmonary resistance parameters using FO is a technique that has been in development for nearly 30 years, but
recent efforts at automating the technique may make it available for wider clinical application. The potential advantages of FO include the ability to measure lung function with minimal patient effort, making testing available in the very young or in patients unable to perform spirometry or plethysmographic resistance tests, and the ability to separate resistance components of the system (upper airway vs lower airway). At their current state of development and validation, these tests do not replace existing pulmonary function tests.

ACKNOWLEDGMENTS: The authors would like to thank Dr. Mark Wylam for reviewing the section on exhaled NO and Audrey Schroeder for preparation of the manuscript.

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