Clinical Application of Pulmonary Function Testing in Horses

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Summary

Pulmonary function tests are emerging as an essential tool in equine referral practice. Indications include intermittent cough, excess mucus, abnormal breathing pattern, presence of wheezes or crackles on auscultation, or exercise intolerance. Since non-infectious airway obstruction and inflammation are the most common problems encountered in the lower respiratory tract of horses, tests are largely aimed at describing the severity, anatomical pattern, stability (reactivity) and reversibility of airway constriction. A comprehensive approach often requires multiple tests, since the information gained from each method is different. Four important tests of mechanical function are discussed in this chapter. They include (1) conventional lung mechanics using pleural pressure measurements, (2) forced oscillatory mechanics, (3) flowmetrics ("boxless" plethysmography) and (4) forced expiratory maneuvers. The implementation of bronchoprovocation to assess airway reactivity is reviewed. The flowmetric test can be employed in the field, while the other methods are suitable for specialists and researchers in the pulmonary laboratory. The importance of pulmonary function testing to the early diagnosis of lower airway obstruction is emphasized, since this is the widest application.
1. Introduction
The respiratory system does not possess the adaptability of other body systems to training, such as the heart and skeletal muscles [1,2]. Compensation for minor disease is therefore limited, especially during high intensity exercise where there are superimposed mechanical and locomotory constraints on respiration. Due to the subtleties of respiratory disease, lung function tests are emerging as important diagnostic tools in the evaluation of performance problems in the equine athlete.

With the recent view that lung disease is widespread and impacting significantly on performance in horses [3], there is a strong need for screening tests that address these problems. Typical respiratory signs that prompt these investigations include chronic or intermittent cough, excess secretions, or exaggerated respiratory rate or effort during exercise or recovery. Lung function tests are used to determine the functional significance of these specific respiratory signs in particular to differentiate obstructive from non-obstructive conditions [4]. In horses with more overt signs of lower airway obstruction (i.e., heaves), lung function testing has a different role. They are used to assess disease severity, bronchodilator effects, breathing strategy, and the basis for refractory cases. More recently there is growing interest in the early detection of lung disease in horses with poor performance, which do not exhibit respiratory signs. In this scenario, one is responding to trainer or rider observations of reduced performance, such as slowing at the 3/4 mile point in racehorses, reduced training times, difficult recovery from exercise, poor impulsion, heat intolerance, and refusal of activities. Highly sensitive lung function tests can help detect lung disease in these competitive horses and aid in developing a unique strategy for treatment where there are drug restrictions. Lung function tests can be persuasive to clients that need to make major environmental changes. Finally, lung function tests can be employed in the pre-purchase arena, although this is a new application that is relatively unexplored.

Until recently, lung function tests have been employed almost exclusively for research. There is a practical difference between clinical and research testing, as the latter allows more freedom to perform difficult or more invasive tests. Although there are several excellent papers that discuss how to proceed with selected tests primarily for research purposes [5-8], the application to clinical patients requires a new approach and a comprehensive review. A programmatic approach to lung function testing, including surveillance, serial testing, and retesting will certainly evolve [9-11]. In this chapter, we will concentrate on those tests that in the author's view, are most suitable for outpatient testing and inclusion in a program for diagnosis and management of obstructive lung disease. The reader will soon discover that no single lung function test addresses all the needs of a clinician. To that end, the physiologic background and technical aspects of four selected systems that measure lung function in different ways (conventional mechanics, oscillometry, plethysmography, and forced expiratory maneuvers) are discussed.

1.1. Specific goals of clinical lung function testing:

1. Detection and quantification of lung dysfunction.
2. Localization of respiratory problem
   (airways vs. parenchyma, upper vs. lower airways, central vs. peripheral airways).
3. Sorting out deranged breathing patterns and basis for respiratory failure.
4. Measured response to rapid onset medication.
5. Measured response to long-term medication.
6. Assessment of "best" lung function.

In order to sift through the available tests, it is important to understand the nuances of each. The first consideration is the invasiveness of the test. Clinical tests must be applied to horses without prior experiences in the laboratory or clinic setting or with the test itself. In this regard, the special skills of the equine handler are equally as relevant. Non-invasiveness is crucial for serial testing, especially when embarking on serial testing, as is necessary for bronchoprovocation, bronchodilation.

5. Forced expiratory maneuvers and lobeline-induced hyperpnea
   5.1 Physiological basis for forced expiratory maneuvers
   5.1.1 Methods described for forced expiratory flow - volume maneuvers
   5.2. Lobeline induced hyperpnea - an alternative method for studying flow limitation

6. Bronchoprovocation testing - tests of non-specific airway reactivity
   6.1. Introduction to bronchoprovocation
   6.2. Theories behind the pathophysiology and genesis of airway hyper-reactivity
   6.3. Methods of bronchoprovocation
   6.4. Clinical data in horses - normal, inflammatory airway disease (IAD), and heaves susceptible

7. Conclusion
deflation, constrained at this level by the outward recoil force of the chest wall. Again, this requires patient cooperation and is
dilution [13] or nitrogen washout [14,27]. These methods are less useful in horses with airway obstruction, which acts to
outward), with the breathing muscles in a relaxed state. Measurement of FRC is feasible using methods such as helium
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maximum inflation volume, and this is not a dependable measurement during tidal breathing, exercise, or hyperpnea.
hold and is therefore not possible in horses. It is a static lung volume that requires equilibration of pleural pressure at
maximum inflation. Recoil of the lung and chest wall prevent further chest expansion. This measurement requires a breath
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restrict gas communication between alveoli and airway opening. Residual volume (RV) is the lung volume at maximum
deflation, constrained at this level by the outward recoil force of the chest wall. Again, this requires patient cooperation and is
not measured in the horse during tidal breathing. However, under anesthesia, RV can be estimated if FRC is known, by
subtracting the gas that can be removed from the lung by suction to a given pressure. It is difficult to define the point of RV,
i.e., often too much air is removed, and the results are not physiologic. Furthermore, even this simple method is obfuscated
by the premature closure of airways, so actually estimates "closing capacity". Hence, "true" RV can not be measured in the
awake horse.

1.3. Static tests that defy measurement in the awake horse
Tests that are performed with the respiratory system at equilibrium and zero flow, are referred to as static tests. Examples
include measurement of lung volume subdivisions and compliance of the lung and chest wall.

1.3.1. Lung volume subdivisions
Lung volumes are simple to understand and common descriptive currency in the clinics, however, most lung volume
subdivisions can not be measured in awake horses. Total lung capacity (TLC) is the total gas in the lung at the point of
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1.3.2. Static compliance and passive lung and chest wall pressure-volume characteristics.
As for static volumes, measures of elastic recoil or compliance require breath holds. Although these measurements are not
practical in the equine patient, they are important background to the understanding of lung function, and have application to
the anesthetized horse. Static compliance in horses has been measured only under anesthesia [27-31] or in trained
tracheostomized ponies that are made apneic by hyperventilation [32,33]. For static lung compliance, transpulmonary
pressure (airway - esophageal pressure), and for chest wall compliance, pleural pressure (esophageal - atmospheric pressure)
are plotted against lung volumes during steps (4 sec) of deflation starting at a specified transpulmonary pressure (e.g., 30
cmH2O). A simple endpoint obtained from this maneuver is the chord compliance, which is the slope generated by a series of
pressure-volume points over transpulmonary pressures < 10 cmH2O [28]. Alternatively, the shape of the entire pressure-
volume (PV) curve can be examined using the methods of Salazar and Knowles [34] that models the pressure-volume curve
as a first order exponential as follows:
2. Conventional pulmonary function testing using esophageal pressures

2.1. Dynamic tests

In contrast to static tests, tests that are performed with the respiratory system in motion are referred to as "dynamic". The respiratory muscles set the system into motion, and the elastic forces of the lung perpetuate those forces, for example during expiration. An example of a dynamic measurement is resistance, a measurement that requires flow. This is because resistance arises from friction of air molecules against airway walls. In the absence of flow, we can not "sense" friction. It is the comparison of drive (pleural pressure) with flow that characterizes resistance. In other words, flow is determined by respiratory muscle activity to produce intra-thoracic pressures, and the mechanical properties of the lung (e.g., resistance) that may oppose flow. Flow is influenced by voluntary and involuntary drive, as is resistance. Some dynamic measurements are derived directly from flow, without consideration of respiratory drive or pressures. In these tests, flow limitation is assessed. Examples include forced expiratory volumes (FEV), flowmetric (plethysmographic) variables, and bronchoprovocation as a method to induce flow limitation.

2.2. Pressure-flow-volume measurements used to quantify static or dynamic lung function

Lung function tests examine the dynamic or static properties of the respiratory system in terms of a driving force (pressure) and flow. Lung function per se is based on the relationship between these factors, as well as their independent assessment. In some cases the relationship between pressure and flow is linear and simple, and in other cases it is non-linear and requires complex modeling. For example, measurements of resistance and compliance require simultaneous recording of pressure, flow, and volume (derived from the integral of flow) and these must be related to each other using a mathematical model, to be discussed.

Lung function tests differ depending on the source and location of pressure and flow, and depending on the physiologic setting (rest, exercise, forced expiratory maneuvers, hyperpnea, challenge conditions), the data acquisition system, post-processing methods, and potential for measurement errors. Each method of lung function testing provides new insight and answers new questions, allowing the interpretive landscape to broaden.

The individual components (pressure and flow) and their interactions are all considered lung function tests. Measurement of individual variables (pressure, flow, and volume) during spontaneous breathing is necessary to assess ventilation, in terms of tidal volume, minute ventilation, peak flows, and respiratory frequency. Ventilatory measurements alone have also been useful to describe maturation of the lung in horses [27,36,37].

Pressure data, usually in the form of transpulmonary (pleural versus atmospheric or mask) pressures, are a measure of respiratory muscle work, resistance, and lung volume. In general, pressure or flow derived parameters (e.g., volume) taken on their own, do not sufficiently define lung function. For example, tidal volume measurements do not establish the presence or absence of airway obstruction. In severe obstruction, tidal volume can increase due to increased respiratory drive, or decrease due to flow limitation.

Perhaps a more sensitive approach is to examine dynamic features of flow, i.e., the flow time profile. Flow limitation is evident on flow-time traces as a dip in peak flows. However, these are better examined in the form of flow-volume loops.

Significant changes can be observed in horses with severe heaves [38]. The flow-volume loop shows a characteristic "ski jump" (i.e., concave) deflation segment, decreased expiratory time (Te), and increased peak inspiratory (PIF) and expiratory flows (PEF), increased breathing frequency (f) and minute ventilation (MV), and restricted flows at latter points of expiration (12.5% of tidal volume) when the condition was severe. These horses were acutely affected, so it is possible that chronically affected horses would exhibit a different pattern. However, flow-volume loops can be useful under conditions of severe flow limitation.

Intra-thoracic pressure is similarly affected by pathologic and non-pathologic (thermal, metabolic, stressful) conditions, so must be interpreted with caution if not compared with measurements of flow. Measurements of transpulmonary pressures have utility for monitoring horses with obvious clinical signs of heaves, or to include or exclude horses in research studies [6,32,39-41]. The major determinants of $\delta \text{Ppl}_{\text{max}} < 45 \text{ cm H}_2\text{O}$ in horses with heaves, include resistance, dynamic compliance, and flow rates; in horses with $\delta \text{Ppl}_{\text{max}} > 45 \text{ cm H}_2\text{O}$, further changes in $\delta \text{Ppl}_{\text{max}}$ are more a function of dynamic
compliance and flow rate than resistance [38]. Hence, breathing strategy influences pleural pressure measurements in horses. In sum, it is best to describe the mechanical properties of the lung by consideration of both pressure and flow together. Lung mechanical tests examine the magnitude and time sensitive (phase) relationships between these variables.

2.3. Model of the dynamic respiratory system: The equation of motion

All measurements of lung function that employ more than one variable (e.g., resistance or compliance) rely on a mathematical model for their measurement from pressure and flow. Your assessment of pressure-flow data is only as good as the model or the method to compute endpoints such as resistance and compliance. If the model is too simple, the data will not make biological sense. The model should account for all factors that influence the relationship between pressure and flow, and allows for the measurement of static or dynamic properties. In order to invoke a model that accounts for dynamic properties, it has been customary to use an equation of motion. Before showing the equation of motion, there are certain key points to understand. First, it is important to break down lung function tests into input (pressure) and output (flow). Flow results in the displacement of volume. Volume is the simplest unit, flow is the rate of volume displacement, and acceleration is the rate of flow as follows:

\[ \text{Volume (V)} \]
\[ \text{Volume relative to time, or velocity, is Flow (V') or } \frac{dV}{dt} \]
\[ \text{Flow relative to time, or the velocity of flow is acceleration (V''), or } \frac{dV'}{dt} \]

Volume, flow, and acceleration are influenced by independent characteristics of the respiratory system. This concept is a central to the understanding of the model of lung function, the equation of motion. Volume of inflation is largely limited by static features of the lung and chest wall (compliance = 1/elastance). Flow is limited by friction (viscous forces); therefore, the instantaneous pressure required to generate steady flow is largely defined by resistance. Acceleration of volume, such as occurs at the beginning of a breath in exercising animals, is limited by the inertance of the respiratory system, e.g., the pressure required to mobilize a column of air in and out of an open system. This is a simplistic view without regard to interactions between compliance, resistance, and inertance, but is relevant to all forms of mechanical tests.

To understand how motion (volume displacement) is constrained in the respiratory system, we need to consider how these forces act in concert, by putting them together in a model:

\[ P = \frac{V}{C} + RV' + IV'' \text{ or } P = EV + RV' + IV'' \]

where Elastance – E (1/compliance – 1/C), resistance - R, and inertance - I, act in series to affect pressure (pleural, alveolar) that is applied to volume, flow, and acceleration.

This equation is very useful to see how pressure is affected by 3 independent, but highly interactive forces, each with a strikingly different mechanical basis. The basis for compliance (elastic forces) is quite different from resistance (frictional forces) which is different again from inertance (movement of an air column). We will return to the equation of motion, but first we will make a distinction between static and dynamic measurements of compliance.

2.4. Dynamic compliance

Dynamic compliance can be measured in such a way as to minimize the effects on pressure due to flow. In this way, resistive effects on the pressure-volume relationship are minimized. To do so, pressure and volume are measured during points of zero flow, i.e., the end of expiration and inspiration [42]. The equation of motion for the respiratory system is then employed to compute \( C_{\text{dyn}} \):

\[ P_{\text{lung}} = P_{\text{compliance}} + P_{\text{resistance}} + P_{\text{inertance}} \]

The pressure required for each component is expressed as a volumetric function of compliance ("elastic pressure"), resistance ("viscous pressure"), and inertance of the lung, rewritten as follows:

\[ P = \frac{V}{C_L} + RLV' + ILV'' \]

At points of zero flow, there is no contribution to Pressure from Resistance, and the contribution from inertial pressures are negligible (except at high lung volumes or rapid breathing frequencies), so the equation of motion can be truncated:
\[ P = \frac{V}{CL}, \text{ or} \]

\[ CL = \frac{V}{P} \]

Dynamic compliance can be measured as the change in volume divided by the pressure drop at points of zero airflow [43] (Fig. 1):

\[ C_{dyn} = \frac{\delta V}{\delta P} \]

This method has been widely applied in horses [5, 41, 44-48]. It is important to understand, however, that \( C_{dyn} \) is influenced by both static (elastic) and dynamic (obstructive) properties of the lung. Obstruction in the periphery in particular, results in elevated maximal changes in transpulmonary pressures relative to changes in lung volume. This may be explained by a heterogeneous pattern of bronchoconstriction in the small airways. Horses with severe interstitial disease [49, 50] exhibit low dynamic compliance, as do horses with severe small airway obstruction such as that found in recurrent airway obstruction [46, 51, 52] or during histamine challenge [41, 48, 51]. Proper interpretation of lung function requires simultaneous measurement of resistance (\( R_t \)), which, in contrast to \( C_{dyn} \) is largely a measure of frictional (viscous) events found in the large (central) airways [7].

2.5. Pulmonary resistance

Resistance is a measurement entirely dependent on flow. Without flow, there would be no opportunity to measure resistance, since resistance is the relationship between viscous pressure and flow. Resistance of the airways (\( R_{aw} \)) plus lung tissue (\( R_t \)) is referred to as pulmonary resistance (\( R_t \)), which can be measured using transpulmonary pressure (esophageal or pleural vs. airway opening pressure) and flow. Airway resistance along (\( R_{aw} \)) must be measured using a system that separates the contribution from airways and tissue, such as oscilometry (see below), or by measuring the pressure drop across the airways, using an alveolar capsule, for instance. The measurement of pulmonary resistance using transpulmonary pressure (esophageal minus airway opening pressure) is conventional in the horse. The computation of pulmonary resistance \( R_t \) may employ a two-point approach as for dynamic compliance, except that increments of pressure and flow instead of pressure and volume, are measured. The equation of motion is once again implemented:

\[ P = \frac{V}{CL} + RLV' + ILV'' \]

When this equation is applied to the respiratory system during quiet tidal breathing, where inertance (\( IL_t \)) is negligible [52] it can be simplified as follows:

\[ P = \frac{V}{CL} + RLV' \]

A major confounding influence on resistance is lung volume, since airways are opened to a greater extent with inflation ("tethering" or "airway-parenchymal interdependence"). This is probably the greatest weakness to the measurement of pulmonary resistance using conventional measurements, the ever-shifting lung volume. Furthermore, compliance decreases with volume at or near maximum inflation, so elastic pressures become important contributions to total pressures exerted by the horse.

In one method, transpulmonary pressure is measured at two points of equal lung volume (above baseline), one during inspiration and the other during expiration [42]. This gives rise to the name "isovolume method" of calculating resistance from a strip chart data (Fig. 2).
As the change in volume is zero, the change in pressure is related only to resistance, and the equation of motion is once again simplified as follows:

\[ P = RL V' \text{ or } RL = P/V' \]

Hence, resistance can be measured as the pressure shift between two points of equal volume, divided by the absolute difference in flows across these two time points. The flows employed at equal volume points (usually 50 to 70% of tidal volume) usually correspond to the plateau flow. The measurement spans inspiration and expiration and therefore weights each segment equally. Worsening of resistance as in heaves, is apparent as an increase in the increment of pressure relative to flow (Fig. 3).

Figure 3. Pulmonary resistance as measured by the iso-volume method increases in horses during heaves crisis due to increase in the \( \delta \) Ppl and decrease in the absolute change in flow, measured at two points of equivalent volume. - To view this image in full size go to the IVIS website at www.ivis.org.

Resistance can also be measured by subtracting elastic pressure from the total pressure, observed on a strip chart [45] or by plotting pressure and flow on an X-Y scale, and determining the slope. This method also requires subtraction of elastic pressure using an analog circuit as described by Mead [53]. The X-Y plotting is also useful to discriminate inspiratory from expiratory changes in pressure-flow and pressure-volume data. However, these methods are more of historical interest, since automated methods employing commercial data acquisition software that make computations of whole breath-by-breath mechanics have largely surpassed manual computations.

2.6. Sensors used for conventional measurements: Flow, volume, and pressure

Sensors are defined as the recording interface in contact with the animal and associated transducers and cables. Sensors utilized for conventional mechanics include those that measure volume displacements (pneumotachographs, spirometers) and pleural pressure (esophageal balloon catheters) and their respective differential pressure transducers. The most important characteristics of sensors is their (1) ease of calibration, (2) stability of balance, (3) excellent frequency response, (4) consistent amplitude response, (5) linearity over span of physiologic measurements, and when used in conjunction with other sensors, their (5) relative phase matching. Measurement of pleural pressure involves the insertion of an esophageal balloon catheter that is long enough to reach the midthorax [6]. In this way, thoracic esophageal pressure is used to estimate pleural pressure [53-57]. The balloon can be coupled with a transducer that is located outside the horse or at the tip of the balloon. The latter system affords a faster frequency response. If using a simple balloon tip catheter with a transducer external to the horse, the tip of the catheter tubing is modified by puncturing holes in a spiral arrangement [6,28]. Latex condoms are frequently employed for the balloons, as they are thin walled (0.05 - 0.1 mm) and highly compliant; a 10 cm segment of balloon is tied off above and below the holes. Patency of the balloon is checked by inflation under water. The catheter is stopcock and allows one to fill the balloon with a small amount of air (e.g., 3 mL). The exact volume must be determined by examining the working volume of the catheter [55] over which there is no increase or decrease in resting pressure of the system.

The sensor (catheter tubing and transducer) prior to attachment of the balloon catheter is calibrated under static conditions, using a simple water manometer (U-type or slant) over the expected range of the subject (0 - 20 cm H2O, or 0 - 60 cm H2O for horses with heaves). Generally, one should employ maximum calibration pressures and amplifier gains that result in approximately one half the maximal voltage output. This would provide an acquisition system with good signal to noise ratio, avoidance of transducer overload and analog to digital converter saturation - i.e., a plateau in signals.

The distance you need to pass the esophageal catheter can be pre-determined by putting the catheter on the horse, with the balloon tip positioned at mid-thorax (intercostals space 8 - 10), and marking the point where the catheter exits the nares for reference. If the catheter is properly lined up along the pathway of the esophagus, the distance will generally be accurate. One can verify position by occluding the mask and examining a neutral pressure response, since pleural and alveolar (airway opening) pressures during occlusion should be nearly equal. Looping indicates poor positioning or poor frequency response of the catheter.

Insertion of the esophageal balloon catheter through a guide tube is helpful, for example the use of a 1/2 length stomach tube. However, some investigators do not use a guide tube. If you do, the esophageal balloon catheter is positioned within the lumen of the insertion tube. Once the tip of the insertion tube is located in the distal cervical esophagus, esophageal balloon catheter is pushed through while the guide tube is removed. The esophageal catheter is advance further until the balloon lies in the thoracic esophagus. Alternatively, the catheter can be inserted within the guide tube to the stomach, where positive
pressures will be found in relation to inspiration. The guide tube can be removed, and the catheter withdrawn into the thoracic portion of the esophagus, for pleural pressure estimation. Adjustments in the position of the catheter may be necessary in order to obtain a maximum pressure deviation. Maximum difference in transpulmonary pressure ($\delta P_{plmax}$) is usually defined as the maximum between points of zero flow. If one is only interested in the measurement of $\delta P_{plmax}$ and not measuring flow, one may define $\delta P_{plmax}$ as the maximum change in pressure during the breathing cycle. There are many references to this method in equids [6,28,29,40,45]. Lateral tracheal pressure can be substituted for mask pressure if one desires to exclude upper airway resistance, which ranges 50 - 70 percent of total pulmonary resistance at rest [58].

Flow is measured using a pneumotachograph, such as a Fleisch type [32,36,37,45,59,60], screen type, or ultrasonic device [26,61], all which have excellent frequency responses and linearity over the physiologic ranges of horses during quiet breathing [62]. Calibration of flow can be performed by forcing a known volume through the system using a precision syringe or by introducing a steady state of a known flow using a rotameter as a reference. Obviously calibration at high flows, compatible with exercise, requires special consideration, since peak flows can reach 100 L/sec 1, (see T. Art and W. Bayly vida infra). Probably the biggest problems associated with pneumotachographs include (1) obstruction of sampling ports with condensation or saliva, (2) moisture accumulation on the pneumotach elements which changes the linearity and calibration of the device, (3) the added resistance of the pneumotach which changes breathing pattern, and (4) imbalance of the flow transducer causing drift.

Tidal volume is obtained by electronic (analog or digital) integration of flow. Hardware based integrators have a tendency to drift, hence the preference for software integrators. For automated breath by breath analysis of conventional parameters it is customary to define an acceptable breath for analysis, usually by setting up inclusion criteria ("filters") in the software. For example, in adult horses (500 kg), inclusion criteria might be as follows: tidal volume > 2 L, inspiratory flow >1.0 L/sec, inspiratory to expiratory volume ratio 0.7 - 1.3, inspiratory time > 0.5 and < 5 seconds, frequency between 5 and 30 per min, and $\delta P_{plmax}$ > 3 cm H2O. These criteria tend to exclude sniffing, coughing, low-volume breaths, and major shifts in FRC during tidal breathing. A common problem in conventional testing is the presence of esophageal contractions that obfuscate pressure measurements - one may need to manually exclude breaths overlaid by esophageal contractions, one reason that it is essential to review pressure and flow traces manually. This limits the real-time value of the information in clinical practice. If frequency response and amplitude characteristics were perfect for all sensors, there would be no concern with phase lag between sensors. However, frequency responses are not perfect, largely due to the lag effects of catheters and downstream connectors and tubing. Transducers available today (e.g., Validyne) have near perfect frequency responses. To minimize amplitude dampening at higher frequencies, one should minimize points of constriction (e.g., too many connectors) and use tubing with minimal compliance. To avoid phase mismatching, tubing should be as short and non-compliant as possible, and length of tubing on each side of the transducer and for each sensor, should be equal and free from obstruction, e.g., condensation or saliva. The screen or mesh of the pneumotachographs must also be clean and their ports free from debris or condensation. The frequency response of the esophageal balloon plus catheter, each post of the pneumotachograph, and the pneumotachograph ports when combined should be checked [6,45]. Phase matching should be checked whenever there are changes to your sensors or downstream connections. This is achieved by applying oscillatory, or step frequencies of pressure (within a closed glass bottle or tube) to the sensors [6,45,58], and measuring the frequency and amplitude responses. Lavoie [58] found excellent phase matching between cannulas used for lateral tracheal or lateral mask pressure recordings and respective esophageal balloon catheters at low frequencies (20/min) using sinusoidal flows. Factors that increased phase lag included faster frequency, resistance, and parallel (rather than perpendicular) positioning of pressure ports relative to the gas flow. Amplitude recordings became more variable at higher frequencies, considerably above those typically measured in vivo.

Due to the effects of frequency on phase matching and amplitude responses, the test should be performed at up to five times the expected frequency of the subject, and mismatch of less than 10 degrees should be obtained, and preferably < 5 degrees mismatch or loss of amplitude in either sensor.

Phase lags are common when using tracheal catheters, as they are prone towards accumulation of secretions. Long esophageal balloon catheters may also introduce a lag, and this is compounded by the use of multiple connectors, stopcocks, with different diameters. Excessive tubing compliance, decreased esophageal compliance, will slow the response of the balloon-catheter sensor to rapid changes in pleural pressure. Some systems permit the electronic correction of phase by introducing a time delay in the pressure signal.

### 2.7. Confounding effects of the facemask, pneumotachographs, head position, and sedation

The measuring devices may affect breathing pattern and therefore the results of lung function tests. Compression of the nares with the facemask must be avoided. The development of nasal edema observed with sedation must also be prevented, by keeping the head upright at all times. Dead space less than 10% tidal volume and resistance caused by typical pneumotachographs are minor problems for conventional testing at rest, but can pose serious constraints to testing during exercise [63,64]. Horses appear to alter their breathing pattern when the mask is placed on the muzzle even at rest, by
increasing tidal volume and reducing frequency (unpublished data), a reflection on increased respiratory drive. This may be an effect of increased resistance, dead space, or trigeminal nerve stimulation from the mask (as seen in infants). Significant pressure swings can develop in the mask so it is important to use lateral pressures within the facemask, rather than atmospheric pressure, for comparison with esophageal pressure in order to measure total pulmonary resistance (Rl). Otherwise, the resistance of the pneumotachograph, which may vary with flow, will be included in this measurement. During dyspnea for instance, esophageal pressures (vs. transpulmonary pressures) will overestimate resistance or the drop in dynamic compliance.

The success of pulmonary function testing in the clinics is largely determined by owner-patient acceptance. Horses that are unfamiliar with the clinic and pulmonary laboratory, after 1 - 2 hours in the trailer, can have a level of anxiety that does not permit testing without sedation. Sedation is especially necessary if testing is prolonged (> 15 min) as for bronchoprovocation. The improved restraint must be balanced against the potential confounding effects of the drug. Sedation alters ventilation [65,66]. Xylazine (1.1 mg/kg IV) caused an initial decrease in tidal volume and minute ventilation, with a rebound in tidal volume by 5 min and progressive increase in tidal volume for approximately 45 min post-injection [66]. Rate and minute ventilation remain depressed for up to one hour at this dose. The increased lung volume may cause a reduction in Rt [58]. In addition, there are direct bronchodilatory effects of xylazine, detomidine, and acepromazine [66-68]. Xylazine caused a significant degree of reversal of bronchoconstriction in ponies with heaves [66-68], found similar results for detomidine (10 ug/kg IV) and butorphanol (20 ug/kg IV) in horses with heaves. The bronchodilator effect of xylazine, an alpha-2 adrenergic agonist, results from pre-synaptic inhibition of acetylcholine from parasympathetic nerves, and is reversed with yohimbine [68].

Sedation with xylazine also causes lowering of the head, which increases upper respiratory tract resistance by altering pharyngeal patency and promoting nasal mucosal swelling [69,70]. Both effects are minimized by keeping the head upright [70,71], or by nasotracheal intubation [71]. The search for a non-bronchodilatory sedative for use in pulmonary function testing in horses has been less than fruitful. For example, buprenorphine had no significant effect on Rt, Cdyn, or δ Ppl max in normal or heavy horses, but caused too much excitement [72].

Hence, the use of sedation has a major disadvantage in that obstruction caused by parasympathic stimulation is partially reversed by the time the measurements are made. Whether the same effect is apparent in horses with lesser-grade obstruction or obstruction due to other mechanisms (e.g., geometric narrowing due to wall thickening) is unknown. Our current approach is to use the minimal dose of xylazine necessary, and this ranges from 0.5 - 0.75 mg/kg IV in our hands. Xylazine does not block the effects of bronchoconstrictive agonists (histamine, methacholine), so it is inappropriate for restraint during bronchoprovocation [9,10,73,74]. The degree of reactivity appears not to be influenced by xylazine but this warrants further study. Kuehn [75] found that marked airway reactivity persisted and was highly reproducible even when xylazine was used as restraint.

Diurnal effects on resistance and compliance and ventilatory parameters should be considered [46,76]. The variation in pulmonary function appears to be greatest in horses with heaves, in particular the daily variation in Vt, and the circadian and monthly variation in Cdyn was greatest in horses with heaves. These cycles may relate to temporal fluctuations in inflammatory mediators (e.g., leukotrienes which peak nocturnally), endogenous steroids, autonomic, and non-adrenergic non-cholinergic mechanisms. Hence it is best to make repeated measures at the same time of day where practical.

2.8. Clinical applicability of conventional lung function testing

The conventional method of lung function testing is often viewed as the gold standard for the diagnosis of airway obstruction, even though the sensitivity for low-grade obstruction is at best fair [33,77,78]. Technically this is a rigorous method, and not particularly suited for outpatient or field-testing. The requirement of an esophageal balloon catheter in particular makes this method time consuming and unattractive, especially for clients and horses that are not familiar with the procedure. Occasional epistaxis from the insertion tube precludes testing that day.

The advantages of conventional testing include the (1) direct measurement of pleural pressure, from which resistance, dynamic compliance, and work of breathing can be obtained, (2) a sense of respiratory drive and "pattern" hence breathing strategy by examining the pleural pressure waveforms, and (3) a measure of resting end-expiratory pressure, which results from the balance between lung and chest wall forces (can be increased with air trapping), and (4) large end-expiratory pressures, exceeding zero, indicative of severe bronchoconstriction.

One of the main disadvantages of conventional testing is that the measurement domain is restricted to the spontaneous breathing frequency, a rate that tends to vary little during the test. Hence, frequency sensitive aspects of lung function (i.e., frequency dependence of resistance or compliance) are not recognized. The latter information is necessary to make inferences on serial or parallel heterogeneity of constriction in the lung, an early sign of small airway disease (see section on Forced oscillatory mechanics - Oscillometry). Using conventional tests, frequency dependence of resistance in horses with heaves was noted in one study [79], by correlating respiratory frequencies over a narrow range with resistance, but this required tremendous patience and post-processing efforts, and higher frequencies were excluded from study.
Overall, reports of conventional testing data from "clinical cases" is sparse in the literature [51] despite a plethora of research testing. The imbalance suggests that the tests are more pertinent to research. Part of the reason is that horses get especially restless with the esophageal balloon catheter in place, and many breaths are unusable due to esophageal contractions (large positive pressure deflections). Despite these concerns in clinical patients, conventional testing is still the gold standard for evaluation of new drugs in the USA and Europe. Allergen challenge results in highly repeatable changes in pulmonary resistance, dynamic compliance, and maximum shifts in transpulmonary pressure [80], making the allergen induced heaves model suitable for clinical investigation. Coupled with a clinical scoring system, the use of conventional testing is an appropriate platform for the laboratory stage of drug testing. The availability of clinical scoring systems [77] that correlate with conventional variables, facilitates the transition from laboratory to field trials. It is important in this regard to recognize that conventional testing is only as sensitive as clinical examination [32,77,78], so any study based on this scheme will require the inclusion of moderately to severely affected horses.

3. Forced oscillatory mechanics (oscillometry)

3.1. Introduction to clinical oscillometry

Forced oscillatory mechanics is the study of dynamic lung function by measuring the response of the respiratory to external forces (pressure or flow). The pressure-flow signals from spontaneous breathing are largely ignored in this method. The concepts of oscillometry arose from electrical models that describe impedance. The analogy between electrical impedance and impedance to airflow was first described by Otis [81] then applied to measurement of respiratory function by Dubois [82]. The basis for these models is the notion that the impedance to airflow is the sum of three effects: Resistance (pressure due to friction), elastance (pressure due to tissue recoil), and inertance (pressure related to acceleration of flow). As for conventional modeling using the equation of motion, these three forces act in series. Oscillometry has been performed extensively in humans, for example in the study of airway obstruction [83,84], bronchodilator effects [85], bronchial challenges [86-89], occupational disease manifestations [90], clinical drug efficacy [91], infant lung function [92], and the behavior of tissue and airway components of the respiratory system in normal [93] and during bronchoconstriction [79,94-96].

Among domestic species, the most significant body of work was performed in cattle [97-100] and dogs [101]. The first studies concerning oscillometry in horses were conducted by Young [102-104] and Le Ninivin and coworkers [105], and later by van Erck and coworkers [106], Hoffman [10,73], and Mazan [97], and recently, oscillometry has found its greatest clinical application in awake animals, in the horse.

The conceptual framework of oscillometry is highly sophisticated. There are excellent reviews on the principles and application of oscillometry by Lutchen and Suki [107], Peslin and Fredberg [108], and Pride [109]. Vogel and Smidt produced an outstanding book on oscillometry with an emphasis on impulse oscillometry in humans [110]. Another learning opportunity is a physiology course ("The Pulmonary Mechanic") with emphasis on oscillometry, available on an annual basis in October or November, by Professors Jason Bates and Charles Irvin (Biophysics, University of Vermont), and Kenneth Lutchen (Boston University) at the Respiratory Center, University of Vermont, Burlington, VT, USA (contact Veronica.Gardiner@vtmednet.org).

One does not have to know how to perform the mathematical derivations of impedance to use this method clinically. The user interface of commercial systems is easy to use. Oscillometry has the other advantage of being completely non-invasive. For those who wish to understand the qualitative aspects of oscillometry, consider the following introduction. For more details concerning the quantitative approach, the reader is urged to seek one of the aforementioned references, or attend a course.

3.2. Principles of oscillometry - the measurement of impedance from pressure-flow data

Oscillometry is the study of lung mechanical function by the application of external forces to the respiratory system [82]. An external source of energy, either a loudspeaker or air pressure, is used to impose air currents, directed via facemask, on the respiratory system. The intent is to produce measurable oscillations of pressure, also measured at the level of the airway opening. Simply, an inordinately high pressure output relative to a given flow signifies increased impedance. In addition to the increased magnitude of pressure, impedance is signified by exaggerated phase mismatching between pressure and flow signals depending on the nature of the obstruction or disease. An added dimension afforded by testing with oscillometry is the control of input frequency, as opposed to the lack of control during conventional testing. It is precisely this control of forcing frequency that provides additional insight into the respiratory system. In addition, one can control the input energy (e.g., amplitude of flow), and the exact waveform (sinusoidal, quasi-sinusoidal, square) that is used. Oscillometry inputs may be single frequencies ("monofrequency forced oscillatory system", FOS) [103,104], multiple frequencies fed one after the other ("multiple frequency oscillometry system", MFOS) or simultaneously in a random assortment ("random noise") [102] or carefully controlled montage ("optimum waveform ventilation") [111]. In addition, a single impulse generated from a loudspeaker in response to an electrical squarewave, can be introduced into the respiratory system and responses measured across multiple frequencies (impulse oscillometry system, IOS) [106].
Testing over a range of frequencies allows a more sensitive measurement of airway caliber, and provides insight into the pattern of constriction in the lung. The major patterns of constriction are homogeneous and heterogeneous, central and peripheral. Heterogeneity is a term applied to the spatial/geometric characteristics of constriction [79,107,112]. When impedances are detected in both central (large or upper) and peripheral (small) airways in the same animal, constriction is in series, as opposed to constriction in airways of similar generation (in parallel). When impedance is detected in airways of similar generation, constriction is in "parallel", and the frequency dependence of the respiratory system is increased. Series and parallel impedances are forms of "heterogeneity" – as opposed to homogeneous constriction. Heterogeneity is characteristic of inflammatory airway diseases such as asthma in humans [96] or inflammatory airway disease (IAD)[4,9,10] and heaves [80,103,104] in horses. Oscillometry is used to series and parallel impedances, by examining the behavior of impedance and its components (in particular, resistance and elastance) as a function of frequency. The pattern of constriction relates with disease severity, stability, and etiology. For example a young horse with heterogeneous peripheral airway constriction can be diagnosed with IAD. A horse with central airway resistance and without peripheral airway disease may have upper airway or tracheobronchial disease which is unlikely to respond to treatment for IAD.

The principles of resistance, dynamic compliance, and inertance and their origins from pressure-flow, pressure-volume, and pressure-volume acceleration still apply to oscillometry. The magnitude and phase of pressure and flow are still the principle signals from which resistance, compliance, and inertance are derived. Because oscillometry employs sinusoidal or similar periodic forces to excite the respiratory system at a range of frequencies including those that are supra-physiological (> 2 Hz), the phase relationship between pressure and volumetric displacement becomes extremely important. Whereas inertance is unimportant during spontaneous tidal breathing at rest [113] it contributes significantly to oscillometric measurements. The high frequency of testing is also technically more demanding of sensors. There are distinct advantages to modeling and therefore analyzing pressure-flow data when sinusoidal signals are applied [108]. For one, the respiratory system can be modeled using electrical theory, with electrical resistance, capacitance, and inductance, as analogues of resistance, compliance, and inertance, respectively of the respiratory system. Oscillometry therefore gives us the opportunity to mathematically, and graphically, model the respiratory system because the assumption of linearity and sinusoidal signals can largely be satisfied when testing horses, at least those without severe disease.

3.3. Oscillometry hardware - external forces and sensors
In the spontaneously breathing animal, one introduces pulsations of flow into the respiratory system via a T-piece (Fig. 4).

The horse breathes through the side port ("T" portion), which is covered with a low-resistance screen. This screen diverts a portion of flow to the respiratory system of the horse. Flow (or pressure) is forced through the T-piece into the respiratory system using compressed air or a loudspeaker as the generator. The waveform and frequency of pulsations is controlled by a sine-wave generator, which activates a proportional valve in the case of compressed air. In the mono-frequency forced oscillation system (FOS), sine waves are introduced one frequency at a time, and the pressure flow characteristics recorded in terms of magnitude and phase for each frequency. (Fig. 5 and Fig. 6).
The advantage of FOS is the ability to focus energy at certain frequencies of interest, simply by testing them individually. For example, low frequencies provide insight into the status of the lung periphery, but require considerably more energy to "inflate" this zone. In the method of impulse oscillometry (IOS), impulses generated with a loudspeaker are delivered to the lung at many discreet frequencies simultaneously. The advantage of such a system is that (1) the energy transferred at each frequency is equivalent and (2) measurement over multiple frequencies is conducted simultaneously. The quickness of the method improves temporal resolution. A potential disadvantage of IOS is that the energy from the impulse is distributed equally to frequencies of little interest. Other methods to force the respiratory system are the use of random noise from a loudspeaker [114,115], a method studied in horses [104], and transfer impedance, measured by oscillating the body surface and recording the pressure response at the pleural surface [107]. The latter system requires the use of an esophageal pressure catheter, and flow is measured at the airway opening.

Both FOS and IOS compare the input (pressure) to the output (flow), events that are measured at the airway opening. The flow sensor has identical characteristics as that employed for conventional mechanics, i.e., a pneumotachograph. Pressure at the airway opening is measured from a laterally positioned port located between the pneumotachometer and facemask. These sensors must have excellent frequency responses. The transducers for FOS (On The Nose®, Scientific Solutions, Loughborough, UK) are identical to those used in conventional testing. The transducers for the commercial IOS (IOS MasterScreen, Jaeger, GmbH, Wurzburg, Germany) are incorporated in the measurement head.

Flow at the airway opening (V’ao) is a reflection of transfer of viscous (resistive) and stored (elastic, inertial) energy that is derived from pressure (P’ao), i.e., a tangible signal that reflects the mechanical properties of the lung, principally the airways. One can not obtain accurate information at the breathing frequency, since the measurement of generated flow at the airway opening (V’ao) cannot be distinguished from spontaneous flow. Hence, frequencies of V’ao exceed the breathing frequency by a sufficient magnitude to avoid such distortion of the input flow signal.

3.4. How oscillometry in anesthetized animals differs from the awake patient - measurement of impedance at frequencies equivalent to spontaneous breathing

The highest fidelity of measurements is only possible in anesthetized, relaxed, intubated animals. In this setting, it is possible to test much lower frequencies (e.g., 0.156 - 1 Hz), which span the normal spontaneous range [94,116,117]. A closed system of mechanical ventilation is used to control lung volumes and maintain mean transpulmonary pressure (optimum waveform ventilation) [79]. With this approach, it is possible to separate pulmonary resistance into its airway and tissue components. This is done by examining the frequency dependence of pulmonary resistance and elastance. Pulmonary resistance (= tissue + airway resistance) is frequency dependent over the range of spontaneous breathing frequencies, due to the frequency dependence of tissue resistance. As frequency of oscillation is increased from 0 to 1 Hz, there is a drop in tissue resistance. In contrast, airway resistance is not normally frequency dependent. Hence, the pulmonary resistance vs. frequency curve appears to have an exponential decay due to the decay in tissue resistance. The degree of frequency dependence is greatly magnified with airway constriction. With pathology, the airway AND tissue components become more frequency dependent. Pathologic frequency dependence per se, over the low frequency range, indicates heterogeneity of airway constriction and correlates with the severity of clinical states [79,94,95,107,112,116,117]. There are mathematical models to describe the frequency dependence of tissue resistance (Gti - viscous dampening) and elastance (Hti - tissue elastance), and allow the estimation of airway resistance [94,116,117]. Low frequency testing requires either anesthesia or breath holding. This is not possible in horses, so low-frequency (0 - 1 Hz) testing has not become feasible in this species. The lowest frequency of testing in the horse is reported to be 1 Hz [4,9,10,73], and interference from spontaneous flow arises at this frequency [9]. So frequency dependence of tissue resistance which are found from 0 - 1 Hz are not measured. Hence, mathematical models (Gti, Hti, Raw) currently available can not be employed for this range of testing. Instead, a rough estimate of frequency dependence is obtained by comparing the lowest frequency impedance (1 Hz) to the impedance at higher frequencies (2 - 3 Hz) [48,104]. It is important when comparing equine data, with that obtained using low-frequency oscillometry in anesthetized animals and in humans.

3.5. Oscillometry in the awake horse

In the awake adult horse, oscillometry typically employs frequencies of 1 Hz or greater, to avoid overlap with the breathing frequency and any data that arise from lower frequency events are filtered out. Valuable information concerning the pattern
of bronchoconstriction is obtained at the lower frequencies (1 - 2 Hz) \([10,48,73,93,96,107]\). Higher frequencies (≥ 2 Hz) provide information concerning airway resistance \( (R_{aw}) \). Hence, to examine the data from low and high frequencies separately or in relation to each other, gives valuable information on the peripheral and central airways, respectively. In performance horses, where lesions can be minimal, a sensitive system that permits detection of airway obstruction is extremely valuable to understand the functional significance of poor performance, cough, or airway mucus.

Testing at each mono-frequency for FOS takes about 10 sec (for a total of 30 - 60 sec), and for complete data collection using IOS, the same amount of time on the horse is required. The evaluation of amplitude is made by examining the average amplitudes of flow and pressure at discreet frequencies, obtained from a Fast Fourier Transformation of the entire frequency spectrum of signals. Many wavelets are averaged to obtain a power spectrum for flow and pressure. The amplitude ratio of absolute pressure to flow values is the impedance \((Z)\), often referred to as the impedance modulus \(|Z|\)). The phase relationship between pressure and flow must also be factored in, and oscillometric data acquisition systems are programmed to detect and measure phase angle at each frequency, and report this information to the user. Hence, the magnitude and phase for \(|Z|\) are the initial computations.

For a single frequency, knowledge of the magnitude and phase relationship provides a measure of impedance. However, measurement of impedance at a single frequency gives us an incomplete picture of the respiratory system. In fact, impedance is a broad term that lumps together all the factors that influence driving pressure. Factors that affect the pressure signal which are in-phase with flow are called resistance. Factors that act out of phase (perpendicular) are referred to as compliance - at low frequencies, and inertance - at high frequencies (≥ 3 Hz). These are familiar terms that we examine in a new light by (1) using an external source of flow (or pressure) as the stimulus, and (2) by modeling the pressure and flow waveforms that result as sine waves. This permits one to examine time-sensitive measurements such as phase angle between the pressure and flow signals. The phase angle is expressed in the time domain, by acknowledging that one sine wave forced into the system is the equivalent of one period. One period can be projected over a circle with coordinates from 0 - 360º. For example, two waveforms (e.g., pressure and flow) that are completely out of phase from each other, would have a phase angle of 180º.

There are other ways to express phase relationships in circular coordinates, such as the translation of 360º into multiples of π, such as 360º = 2π, 180º = π, 90º = π/2. This facilitates a trigonometric approach. The frequency of sine waves can also be analyzed using a rate \( \omega \) (= 2π f), referred to as the angular frequency, whose units are radians per sec.

### 3.6. The significance of phase angle

The nature of the phase lag at low vs. high frequency is predictable and worth considering. At low frequency, flow leads pressure (-90º out of phase) since flow is required to generate pressure against an elastic force (elastance, the inverse of compliance) (Fig. 7).

At high frequencies, pressure leads flow (+90 degrees out of phase), as pressure is required to move flow. A simple physical model proves these principles. If one simply forces sine waves of flow into a wide open-ended pipe, with negligible resistance, we will find that the pipe acts as a source of inertance. The pressure signal leads flow by 90 degrees. In contrast, if we oscillate an anesthesia bag, we will find the opposite phase relation, that flow leads pressure. These physical models have parallels to pressure-flow data in animals. First, if we view the respiratory system as a similar in-series two-element system, i.e., pipe and bag, we will measure a combination of compliance and inertance effects. At low frequencies, the compliance effects will predominate, and at high frequencies, the effects of inertance (movement of the column of air, and movement of the air in and out of orifices) predominate. In addition to the forces of elastance and inertance, the conducting airways of the respiratory system contain some finite resistance, which in a normal lung is frequency independent. At some frequency where compliance and inertance differ by equal quantities (sum is zero), and pressure and flow signals are in-phase, the impedance measured is entirely a consequence of resistance in the system. This special frequency is the resonant frequency and should be noted during testing, as it increases with moderate to severe bronchoconstriction \([10,48,102-104]\). With peripheral airway
obstruction, the elastic element ("bag") begins to stiffen, or decrease in compliance and/or increase in resistance. This is most discernable at lower frequencies when there is sufficient time for oscillations to permeate the peripheral as well as central airways (assuming there is also sufficient energy in the oscillator). Resistance measured at low frequencies (1 - 2 Hz) is a reflection, therefore of peripheral and central airway resistance, whereas measurements made at higher frequencies reflect central (large) airways diameter. Resistance is normally independent of input frequency over the ranges commonly employed for oscillometry testing. However, frequency dependence (drop in resistance with increase input frequency) is observed in horses with peripheral airway obstruction [10,73,104] and with bronchoconstriction brought on by exposure to agonists (histamine, methacholine) [48]. This frequency dependence is the result of heterogeneity in the diameter of parallel airways, as occurs naturally with small airway disease or heaves, or with induced bronchoconstriction (Fig. 8 and Fig. 9).

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3.7. Equation of motion used to develop a model of oscillometry
The first order differential equation of motion that we have alluded to earlier for the lung,

\[ \frac{P_t}{V} = C_l + R_l \times \frac{dV}{dt} + I_l \times \frac{d^2V}{dt^2}, \]

can be rewritten to reflect that the pressure differential we measure in oscillometry, across the entire respiratory system (RS):

\[ \frac{P_{RS}}{V} = C_{RS} + R_{RS} \times \frac{dV}{dt} + I_{RS} \times \frac{d^2V}{dt^2}, \]

A simplified version of the equation, for a low frequency system such as the tidal breathing horse, where inertance is negligible.

\[ \frac{P_{RS}}{V} = C_{RS} + R_{RS} \times \frac{dV}{dt} \]

During forcing, pressure associated with compliance is out of phase with flow, and pressure associated with resistance is in phase with flow. To bring together the equation of motion for utility in oscillometry, it is useful to consolidate the volumetric
terms (V and V''), expressing both quotients in terms of flow (V').

At this point, the complexity of mathematics increases to facilitate the handling of cumbersome numerical expressions. An imaginary number, symbolized j, is introduced to permit differentiation (multiplying by jω) or integration (divide by jω). However, imaginary numbers follow a series of rules that are complex and beyond the scope of this chapter. Practically speaking, it is perhaps relevant to know one or two rules. An assumption about imaginary numbers is that $j = \sqrt{-1}$. One $j$ is $90^\circ$ or $\sqrt{-1}$, $j^2$ is $180^\circ$ or -1.

Therefore, the relationship between V and V'' can be written in terms of $j$ and the angular frequency - the conversion between a volume and a rate of volume (flow):

$$V = V' / ωj,$$

(in other words, flow divided by $ωj$ is like integrating flow to get volume).

Since $1/j = -1$ (from $j * j = -1$, $1/j = -j$), this can be rewritten as

$$V = jV' / ω$$

Hence, imaginary numbers such as $j$ are used to simplify the subsequent analysis of resistance and reactance.

The equation of motion written for oscillometry,

$$PRS = RRS*V' + V*1/CRS,$$

is rewritten to use V' by substitution for V in terms of angular frequency:

$$PRS = RRS*V' + jV'/CRS*ω,$$

and by dividing both sides by V', we get an impedance, which is similar in units to resistance ($PRS/V'$):

$$ZRS = RRS' + j/CRS*ω$$

We have derived the term impedance (ZRS) for a system forced at slow frequencies, but this will not always be the case. We can use a more general equation that includes an inertance term. We add back the inertance term:

$$ZRS = RRS + j/CRSω + IRSjω$$

The terms ($IRSjω + j/CRSω$) can be lumped together into one term, since these quantities have a common thread - they both constitute values which are out of phase with resistance, but in opposite directions. Lumped together, their effect is termed reactance (XRS). Lumping CRS and IRS into one term XRS gives the following:

$$ZRS = RRS + jXRS$$

If we know the phase angle between our pressure and flow measurements at various frequencies, we can solve for the components of impedance, which are RRS and XRS as follows (Fig. 10):

Figure 10. The computation of resistance and reactance from pressure-flow data is now performed by computers. The concept of these computations is the translation of magnitude and phase data from the pressure and flow signals into a format that permits the application of trigonometric or electrical modeling as shown. The magnitude and phase can be extracted from (1) a stripchart, which in turn can be depicted in (2) circular coordinates, or (3) in turn as a trigonometric relationship. The calculation of resistance and reactance (reactance = compliance and inertance) are made from the trigonometric data. Note that resistance is depicted on the X axis, denoting that it is the in-phase component of impedance ("real" portion) and that reactance is the magnitude of the out-of-phase component of impedance ("imaginary") including compliance (where flow leads pressure) and inertance (where pressure leads flow). The use of the term "imaginary" is confusing and should not be persuade the user that these are imaginary measurements. - To view this image in full size go to the IVIS website at www.ivis.org.
If we want a more detailed analysis of reactance to get \( \text{IRS} \) and \( \text{CRS} \) individually, we need to make pressure-flow measurements at several frequencies, and solve for multiple unknowns. Although this is not something that can be achieved using a hand calculator, modern computers are used for this task. Measurements at multiple frequencies, allow us to examine the zero crossing of \( XRS \), which corresponds to the resonant frequency. Software programs such as On The Nose® and IOS MasterScreen, Jaeger, Germany perform all these operations rapidly, giving clinical data immediately after forcing. This allows one to design provocation tests, since data are nearly available "real time".

The expressions of \( RRS \) and \( XRS \) at varying frequencies are even more meaningful to the clinician if they are displayed graphically, another feature of commercial software programs. Graphical interfaces demonstrate the absolute values of \( RRS \) and \( XRS \), resonant frequency, frequency dependence of \( RRS \), and changes in these parameters with serial testing, including dose - response curves and interpolation of those.

\[ \Phi = \arctan \frac{XRS}{RRS} \]

\[ RRS = |ZRS| \cos \Phi, \text{ and} \]

\[ XRS = |ZRS| \sin \Phi. \]

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3.8. Interpretation of clinical data

3.8.1. Quality of clinical data

Unlike data derived from conventional mechanics, FOS data must be filtered of noise, and quality indices of noise are customarily applied. The signal arising from natural respiratory frequencies tends to interfere with the collection of the signal at the excitation frequency, principally with the flow channel [102]. This interference is avoided by collecting FOS data at higher frequencies. However, the "noise" per se is quantified. The input of flow and pressure are evaluated in a sort of linear regression to obtain a measure of agreement or quality assurance, referred to as "coherence"; an acceptable value is \( > 0.9 \). In horses \((n = 143)\) presented to our clinic for lung function testing, we observed unacceptable coherence values of \( < 0.9 \) for \( RRS \) and \( XRS \) at input frequencies 1, 2, and 3 Hz, in 22/143 (15.4%), 4/143 (2.8%) and 2/143 (1.4%) cases, respectively. Careful sedation and repeated testing can reduce these problems. In all cases, a FOS test was repeated up to 4 times if \( RRS \) was not found at a coherence value \( \geq 0.9 \). Hence, there is a high proportion of horses (15.4%) in which data at 1 Hz were unusable. The drawback is that important data can be lost, although one can still fall back on the data from frequencies with good coherence (2 - 3 Hz). Low coherence at 1 Hz was observed in 7/47 (14.9%) normal horses, 4/44 (9.1%) SAID horses, and 7/42 (16.7%) horses presenting with heaves or a recent history of heaves. So, this problem did not relate to lower airway disease severity, nor was it influenced by upper airway obstruction or head position, age, breed, or gender of the horses. Potential reasons for poor coherence in spontaneous breathers include shifts in lung volume and FRC, nonlinear detection of signals, harmonic cross-talk and overlap, cardiogenic oscillations, gotttic closure, and noise created by the measurement system. Authors have suggested various ways to combat low coherence, including the use of flow signal averaging method [118]. One needs to assure adequate flow is delivered to the subject, the appropriate range of voltage delivered to the proportional valve to maintain a sinusoidal flow signal, and sufficient amplification of the pressure and flow signals for analysis. Reducing the alteration in breathing pattern due to pneumotachs and facemasks is also important. Amplification occurs at the level of the sensor (transducer), amplifiers, and A to D converters. Higher flows may be necessary at lower frequencies, since these are intended to permeate the lung periphery. Since low coherence was most often found at low frequencies (e.g., 1 Hz) [104], it is suggested to change the lowest frequency of testing to 1.5 Hz instead of 1 Hz when there is poor coherence. The author continues to employ the following frequencies: 1, 1.5, 2, and 3 Hz, over 15, 10, 5, and 5 sec respectively.

In examining the baseline data from a group of normal horses \((n = 40)\), defined as those that had no clinical, radiographic, or BAL cytologic abnormalities (i.e., BAL neutrophils < 5%, mast cells < 2%, eosinophils < 0.5%), there was no association between age (mean ± SD 6.5 ± 4.84 yrs, range 2 - 27), gender, body weight, or breed, and baseline (1 - 7Hz) \( RRS \), \( XRS \), or resonant frequency [10]. The coefficients of variation (CV, between subject) for \( RRS \) (1, 2, and 3 Hz) measured in 10 normal horses, were 42.7, 35.7, and 26.8. In contrast, for conventional lung mechanics, the CV (between subject) for \( R_I \), \( C_{dy} \), and \( \delta P_{Pl\max} \) were 56, 31.3, and 38 in 10 normal horses [48] and for \( R_I \) and \( \delta P_{Pl\max} \) ranged 4 - 62% and 2 - 16% respectively in another study [58]. Repeatability for FOS for sequential measurements of \( RRS \) was evaluated in 10 horses with a spectrum of baseline values. The intra-class correlation coefficients \((r^2)\) were 0.83, 0.83, and 0.91 for \( RRS \) at 1, 2, and 3 Hz, respectively [10].

Sedation with xylazine at a typical dose (0.75 mg/kg) caused a mild bronchodilatory effect when measured with the FOS system [119]. This was apparent in the first 5 min of testing, and only at the lowest frequency of testing (1 Hz). The effect lasted 45 min, when \( RRS \) (1Hz) began to ascend back to baseline as the horse woke up. Some of the horses used for this evaluation had higher that currently accepted values for baseline resistance, hence the effect of xylazine on "normal horses"
may require further experimentation. Alteration of head position had similar effects on respiratory system mechanics [119], in this case $R_{RS}$ (1 - 3 Hz), as previously reported in horses using conventional measures [69,70]. The author is unaware of similar studies performed using other sedatives or tranquilizers in the horse. As xylazine has a predictable and transient effect on ventilation and bronchomotor tone, it is the sedative of choice for oscillometry, but it is imperative that the head is kept in mainly horizontal position, by supporting the head with a crutch, for instance.

3.8.2. Low-grade airway obstruction
The rigorous mathematical basis for oscillometry should not dissuade the clinician from applying this system as a clinical tool, as the graphical and digital user-interface of newer commercial systems provide immediate diagnostic value (On The Nose® and impulse oscillometry (IOS MasterScreen, Jaeger, Germany)). Oscillometry should be considered a first-line method for clinical pulmonary function testing in horses if one wishes to evaluate subclinical lung disease, in particular obstructive lung disease in horses. Indications include horses with vague or equivocal respiratory signs such as coughing, mucus observed in the airways, exercise intolerance or poor performance that can not be explained by disturbances in another body system. These are cardinal signs of non-infectious lower airway inflammatory disease (syn: "inflammatory airway disease", "small airway inflammatory disease"). Horses with inflammatory airway disease will exhibit frequency dependent changes in resistance from 1 to 3 Hz (highest at 1 Hz) and airway hyper-reactivity (Fig. 11) [48,73,74].

Figure 11. Clinical data from three groups of horses (Control, IAD, Heaves in Remission) segregated on clinical and bronchoalveolar lavage cytology data. Note that the IAD and heaves groups exhibit frequency dependence of respiratory system resistance, and that their mean values are higher than in controls. This suggests that in the population of horses with airway obstructive disease, both central and peripheral airways are contributing in various ways to impedance. - To view this image in full size go to the IVIS website at www.ivis.org . -

Baseline measurements should be examined for their absolute values and frequency dependence. An elevation of $R_{RS}$ at low frequencies (1 - 2 Hz) or dramatic frequency dependence denotes that the pattern of constriction in the peripheral airways is heterogeneous. If $R_{RS}$ at all frequencies are elevated to a similar degree, one should suspect constriction in the large airways or homogeneity of peripheral airway disease or both. Peripheral (small airway) constriction is usually heterogeneous, so it is wise to think about large airway disease with global increases in $R_{RS}$. In some horses, there is both frequency dependence and an absolute increase in $R_{RS}$ at all frequencies; these horses have peripheral (small bronchi and bronchioles) and central (large bronchi) airway disease. This pattern of constriction is seen with increased severity of asthma in humans [96] and presumably reflects increased severity of obstructive lung disease in horses. Mild airway obstruction as seen in IAD is associated with greater exercise induced hypoxemia [120], airway inflammation [10,78] and airway hyper-reactivity [73,74]. It follows that the functional significance of mild respiratory signs of inflammation or exercise intolerance should be examined carefully using oscillometry or other tests that define frequency dependence or other indicators of early airway obstruction.

There is tremendous value in the re-examination of horses after a period of treatment and/or environmental management. We have noted that many sport horses that improve clinically also demonstrate a drop in frequency dependence and baseline $R_{RS}$ across all frequencies. Airway reactivity must be assessed as a separate issue, since baseline values including frequency dependence severity do not necessarily correlate with airway reactivity. It is not uncommon for horses with mild respiratory signs to exhibit a normal baseline $R_{RS}$ (1 - 3 Hz) but airway hyper-reactivity. Again, this follows the pattern that has been observed in human asthma.

3.8.3. Moderate to severe obstruction
In horses with more obvious clinical signs of lower airway disease there is greater frequency dependence, greater baseline values of resistance across frequencies, and in heaves-susceptible horses, there are increases in resonant frequency. Horses that have high initial baseline values for $R_{RS}$ (e.g., > 1.0 cm H2O/L/sec) or clinical signs of heaves, should be evaluated for their bronchodilator response, rather than response to bronchoconstrictor agonist. Most horses with signs of heaves respond readily to inhaled bronchodilator (e.g., albuterol, 450 mcg). Less frequently, one observes a paradoxical increase in baseline $R_{RS}$ after bronchodilator administration. This paradoxical response may be the effect of the aerosol propellant or albuterol itself. This response has not been noted in the author's laboratory following administration of other bronchodilators or aerosol drugs. However, a thorough study of this effect has not been performed. If albuterol is ineffective as a bronchodilator at doses up to 900 - 1000 mcg, the author then administers ipratropium bromide. Occasionally there is a modest additional benefit with ipratropium bromide but this is unusual. The use of atropine (0.02 mg/kg) to test complete bronchodilation is another option. The advantage of using inhaled bronchodilators in the laboratory is an opportunity to instruct the owners on their proper use and care of inhaler equipment, and to demonstrate their efficacy in a rather obvious fashion.
4. Plethysmographic method of lung function testing

4.1. Introduction to plethysmography in the horse

"Flow-metrics" is a recently developed method that derives information concerning airway caliber from simultaneous measurements of flow at the airway opening (nasal) and body surface (plethysmographic), without use of intrathoracic pressures [121]. In that respect it differs from conventional methods that rely on pleural (i.e., esophageal) pressure measurement, and oscillometry that examines the flow response to pressures forced at the airway opening. In addition, the plethysmographic method employed for flowmetrics (respiratory inductance plethysmography) provides information about breathing pattern (rib vs. abdomen contributions to ventilation), rib-abdomen synchrony, and changes in end-expiratory lung volume. It is a completely non-invasive, portable system. The sensitivity of this system is similar to conventional testing, and there is a very good correlation with flowmetric variables of airway obstruction and R-domain, C-domain, and δ Pplmax. The flowmetric method is generally coupled with histamine challenge or bronchodilator tests in outpatient or field examinations, to improve sensitivity for inflammatory airway diseases.

4.2. Physiologic basis for "flowmetric" plethysmographic method in the horse

The basis for this approach can be found in earlier literature concerning whole body plethysmography. Whole body plethysmography was devised to measure spirometry, and later modified to be used in the measure of specific airway resistance (sRaw) and FRC by Dubois [82]. Boxless plethysmography was introduced by Konno and Mead [122], largely in an effort to describe the separate plethysmographic events of the chest and abdomen. Plethysmographic measurements of volume and flow are distorted relative to spirometry measured at the airway opening, due to the flow-resistive pressure and the pressure associated with gas compression (during expiration) or rarefaction (during inspiration). Consequently, plethysmographically derived flow or volume measurements are found to over-estimate spirometry [123-128]. The magnitude of discrepancies between plethysmographic and spirometric or pneumotachographic volume or flow, was later employed as a potential index of airway obstruction, initially in the setting of double or single chamber plethysmography in animals [129-133]. These physiologists, largely working with laboratory animals, collectively saw the "glass half full", in what was earlier perceived as a plethysmographic artifact. A simple volumetric index of obstruction was derived by Silbaugh [132] and it was the ratio of nasal (V1) to thoracic (V2) flow (i.e., volume displacement), which decreased in conjunction with C-domain following allergen-challenge in guinea pigs. Others [131] showed similarities between V1/V2 and specific airway resistance (sRaw). The use of a simple volume comparison of course ignored time-dependent, i.e., dynamic events that contribute to the difference between nasal and thoracic measurements of flow. Pennock [130] showed a good correlation between phase and magnitude differences between nasal and thoracic volumes in guinea pigs using double chamber plethysmography, in part addressing the time-dependent aspects of gas compression. Later, Sackner [134,135] compared time-dependent plethysmographic (whole body and boxless inductance methods) vs. spirometric data during forced vital capacity maneuvers in humans. Instantaneous flows at different periods throughout the expiratory maneuver measured by box and boxless plethysmography exceed those measured concurrently with spirometric flows at the airway opening, and these changes were more severe in chronic smokers. These observations gave credence to the notion that boxless plethysmography, a method more suited to large animals, may have clinical utility to detect small airway disease if coupled with a forced maneuver or provocation so as to amplify the effects of gas compression and flow-resistance. The question arose as to how to analyze such data. If flow waveforms were sinusoidal in horses, we could employ directly the electrical or trigonometric approach of analyzing magnitude and phase angle between waveforms, but they clearly are not sinusoidal, possessing biphasic and other irregular features [36,136]. Jaeger [123] observed that the phase angle between mouth and thoracic volumes in humans was a function of resistance, compressibility of gas (barometric pressure and volume), and respiratory frequency, defined by the equation:

\[ \tan \Phi = -2\pi f C \]

where \( \tan \) = tangent, \( \Phi \) is phase angle (in radians) between mouth and thoracic volume signals, \( f \) is frequency, \( R \) resistance, and \( C \) is compressibility of gas.

This theory can be applied to plethysmographic measurements of any kind. With increased breathing frequency, airway resistance, and thoracic gas compressibility, there is a greater phase angle. In the case of large animals, where box plethysmography is not generally practical, we can measure the agreement between boxless plethysmography and pneumotachography [121] as a measure of flow resistance and compressibility of gas. Hoffman [121] examined selected regions within the inspiratory and expiratory breath where there were clear differences in pneumotachographic and plethysmographic flow, as a surrogate to measuring phase angle between these signals (it is difficult to examine phase angle in non-sinusoidal signals). Peak and area differences correlated well with conventional variables such as RL and \( \delta \) Pplmax during natural airway obstruction, and conversely, while airway obstruction was relieved with a bronchodilator. These observations were extended to histamine bronchoprovocation. The dose response curves from flowmetrics provided similar
information as those constructed from conventional variables [121], and were highly reproducible (r > 0.98, P < 0.001) between days [75].

4.3. Flowmetric sensors, calibration, and testing method

This method requires a facemask, pneumotachograph-transducer-amplifier for measurement of nasal flow, Respibands® ("66 inches and/or 168 cm) and a respiratory inductance plethysmographic (RIP) interface (oscillator-demodulator) (Ambulatory Monitoring, Ardsley NY, USA) (Fig. 12 and Fig. 13).

Although the hardware and software are available commercially, some assembly, testing and adjustments are necessary in the laboratory or clinic prior use. Frequency responses of RIP and the pneumotachograph, including phase and amplitude fidelity, are excellent [121], so phase matching is not likely to be an issue with this setup.

Prior to use, the sensitivity of the rib cage (RC) and abdomen (ABD) bands are set to equivalent sensitivity, so that excursions of each compartment are unweighted. Two bands are placed so they sit gently but firmly on the horse, one behind the last rib (ABD), and one in the middle of the rib cage (RC), between the 9 - 11th intercostal spaces. Once the RIP bands are positioned and connected with the oscillator-demodulator, the facemask is placed on the horse's muzzle. Lastly, the pneumotachograph is attached.

The RIP system produces an analogue signal for volume from each of the RC and ABD bands, which are summed to give a 2 compartment model of ventilation. The phase relationship between RC and ABD is a measure of thoraco-abdominal synchrony. Techniques to measure phase angle (\(\Phi\)) from X(ABD)-Y(RC) plots (Lissajous figures) have been described [122]:

\[
\sin \Phi = \frac{m}{s},
\]

where the \(m\) is the horizontal width of the X-Y plot at the middle (based on Y-axis or RC deviations), and \(s\) is the horizontal width of the X-Y plot projected on the X-axis (based on ABD deviations). Although there is much interest in the use of phase angle (\(\Phi\)) in humans for measurement of thoracoabdominal asynchrony, it does not appear to adequately grade upper or lower airway obstructions in the horse [137].

The sum of the volume signals RC and ABD is the SUM signal. Movements of the individual bands contribute to shape the SUM signal. The volume signals of RIB, ABD, and SUM can be followed to observe tidal breathing, and breath-by-breath changes in end-expiratory lung volume. For the purpose of flowmetric measurement of airway obstruction, the SUM signal is differentiated into a flow signal that represents net "plethysmographic flow". Plethysmographic and pneumotach flows are subtracted in the same time domain to obtain peak and selected area differences between these signals. The differences represent the severity of gas compression (during expiration) and expansion, i.e., rarefaction (during inspiration). Peak differences during expiration (SFE\(_{\text{max}}\)) and inspiration (SFI\(_{\text{max}}\)) and area differences during expiration (SFE\(_{\text{int}}\)) and inspiration (SFI\(_{\text{int}}\)) are computed breath-by-breath. Typical values for normal awake unsedated or xylazine sedated horses are 2 - 3 L/sec for SFE\(_{\text{max}}\) and SFI\(_{\text{max}}\), and 0 - 0.1 L for SFE\(_{\text{int}}\) and SFI\(_{\text{int}}\). Horses with heaves have values for SFE\(_{\text{max}}\) and SFI\(_{\text{max}}\) of 5 - 25 L/sec, and 0.5 - 0.8 L, for SFE\(_{\text{int}}\) and SFI\(_{\text{int}}\). Histamine bronchoprovocation at maximum, results in a 75 - 150% increase in SFE\(_{\text{max}}\) and a 200 - 500% increase in SFE\(_{\text{int}}\).

Calibration can be performed using several different methods, or the system can be employed in an uncalibrated mode. Pneumotach flow during inspiration is the gold standard, and the SUM tidal volume is normalized to inspiratory volume from the pneumotach. In horses with no signs of airway obstruction where the intent is to perform bronchoprovocation, we can...
assume that the baseline of the subject is "normal", and therefore can be employed as a reference for calibration, i.e., SUM (Vsum) is adjusted to match pneumotach volume. The horse acts as its own control and the percentage change from baseline (e.g., 50% rise in SFEmax) is used as an endpoint for bronchoprovocation. The real-time measure of airway obstruction, allows one to conduct bronchoconstrictive or bronchodilator challenges with safe monitoring and immediate results. The data from the respiratory inductance plethysmography (RIP) when compared further allow one to understand the breathing strategy of the horse. This is especially important when investigating chronic dyspnea that may be associated with bizarre breathing patterns such as paradoxical breathing (phase angle = 180 degrees), diaphragmatic fatigue or even paralysis. Of the systems discussed in this chapter, the flow-metric system is the only one that has been extensively tested in the field (farm, stable) in horses. In one study, horses (n = 21) underwent histamine bronchoprovocation on three occasions, first 3 wks then one year apart. The purpose was to evaluate the repeatability of histamine responses in a stable of well-managed horses where there were no changes in management and no treatment administered to the horses during the study [75]. The results show that airway reactivity was highly repeatable, with correlation coefficients > 0.90 for all horses, and > 0.98 for a subgroup of horses (n = 9) with significant airway hyper-reactivity. This would suggest that both the instrumentation, the algorithm of testing using flowmetrics, and the airway reactivity of the horses, demonstrate good repeatability.

Another important application of the flow-metric method is the non-invasive yet objective evaluation of heaves (RAO). In these horses, airway dysfunction is quite obvious, but there are many instances where quantifying this dysfunction is useful. Examples include the testing of bronchodilator effects in individual horses, the analysis of their breathing pattern, time-dependent studies that require a large number of serial tests (twice per day or more), and for research purposes. The flow-metric method has also been applied to field studies that focus on breathing pattern, in particular thoraco-abdominal contributions to ventilation. We have observed several patterns of breathing in horses with heaves, with various contributions of the diaphragm, reflected in the ABD signal. In some cases, there was little or no ABD activity, or paradoxical motion with the RIB following true flow. This would suggest that respiratory failure is occasionally seen in the horses with heaves as loss of diaphragmatic contraction (ABD movement), and these events can only be recognized from objective study of the breathing pattern using a two compartment system such as RIP.

The most pertinent application of "flowmetric" plethysmography is in bronchoprovocation testing, where the sensitivity of the measurement at baseline is less concerning than the measurement of airway reactivity (see Bronchoprovocation below). For measurements in the field, flowmetric testing provides the least rigorous calibration, and the greatest acceptability from horses due to its non-invasive platform. Anywhere from 10 - 15 horses can be challenge testing (using histamine for example) per day. This offers the possibility of large scale field testing of airway reactivity, and may improve our understanding of this "phenotype" amongst horses exhibiting poor performance and clinical signs of respiratory disease.

5. Forced expiratory maneuvers and lobeline-HCl induced hyperpnea

5.1.1. Physiological basis for forced maneuvers

The notion that forced expiratory maneuvers have a place in lung function testing in horses, stems from human use, and the clinical observation that asthma, chronic obstructive pulmonary disease (COPD), and emphysema in humans and heaves in horses is associated with expiratory flow limitation. Several decades of research and clinical application have established the forced expiratory maneuver as a time-honored standard for clinical testing. The forced maneuver was first used as a simple test to evaluate patients with flow limitation by measuring the maximum exhaled volume in one second [138,139]. The basis for maximal flow was later explored by simultaneous measurement of intrathoracic pressure, flow, and volume (derived from flow) [140]. This was a key study in understanding that above a certain intrathoracic pressure, flow ceases to increase, i.e., it reaches a maximum. The physical basis for this maximum is complex, but is largely related to resistance in the segmental airways and the static recoil pressure of the lung [140-143]. Increase resistance (e.g., COPD in humans) and loss of recoil pressure (e.g., emphysema) limit maximum flows.

The horse was a "stranger" to forced maneuvers until Leith [144] and later Gillespie [31] employed the test in anesthetized, tracheostomized horses, placed in a body plethysmograph. However, it was not until Couetil and coworkers [145] bravely revisited this method, that it was applied to awake, sedated, and unconditioned horses that presented for outpatient evaluation. The facilities and implementation of the test would be challenging to replicate, and the test is slightly more invasive (requiring a nasotracheal tube), but the potential to disclose early airway obstruction is an enormous advantage [78]. In comparison, oscillometry and forced maneuvers allow for early detection of airway obstruction, and conventional and flowmetric tests do not, unless they are coupled with bronchoprovocation. The use of bronchoprovocation to assess airway reactivity is an excellent tool but is not a substitute for actual measurement of airway mechanics prior to provocation.

5.1.2. Methods described for forced expiratory flow - volume maneuvers

Materials and methods are reviewed in the papers by Couetil [78,145]. Essentially, the horse is prepared by sedation with detomidine (0.03 mg/kg IV) and butorphanol tartrate (0.02 mg/kg). A nasotracheal tube (20 mm ID) is inserted to the proximal third of the trachea. The cuff of the nasotracheal tube is inflated to create a seal. After a short period of adaptation,
the horse is mechanically ventilated (FIO₂ = 0.7, VT = 6-8 L, and f = 8/min) for about 10 min, which produces apnea, acclimates the horse to positive pressure inflation, and insures a consistent volume history. Employing a 3-way valve, the lung is then inflated manually (using a 30 L volume anesthesia bag) to approximately total lung capacity, or 30 cm H₂O airway pressure. At this time the valve is switched to divert flow from the horse to a large-volume negative pressure sink (-220 cm H₂O) that powers the rapid deflation. In the early portion of the deflation curve, a plateau in flow is arrived at that relates to the nasotracheal tube characteristics (inertance and resistance), analogous to the effort-dependent portion of the maximal expiratory flow curve. As the lung progressively deflates to about 10% of FVC, a reproducible segment that differentiates normal and obstructed horses emerges, analogous to the effort-independent portion of the maximal expiratory flow curve. Forced expiratory flow (FEFₓ), forced expiratory volumes (FEVₓ), and forced vital capacity (FVC) were determined from these maneuvers, by examining the pressure-time and temperature-time profiles from the center of a reservoir tank.

Couetil [78] has been able to define three groups of horses bases on clinical examination, BAL cytology, and conventional mechanics: (1) normals had no clinical signs and normal BAL, (2) horses with heaves (COPD) had abnormal signs, and abnormal conventional mechanics, and (3) horses with inflammatory airway disease (IAD) had mild signs but normal conventional mechanics, similar to Hoffman [10]. With regard to forced expiratory flow-volume (FEFV) parameters, both IAD and COPD groups differed from normals, denoting that both groups possessed airway obstruction, in conjunction with airway inflammation. This demonstrated clearly the importance of airway obstruction in horses that may fail to be demonstrated by physical examination and conventional testing. Forced maneuvers, therefore, constitute a breakthrough in our understanding of the early development and diagnosis of obstructive airway disease.

Another way to disclose lower airway obstruction using function tests is to perform provocation tests. Couetil [145] also demonstrated that FEFV could be measured repeatedly in horses for the purpose of recording the effects of histamine (or other) aerosols in a challenge test. The difference in airway reactivity measured by FEFV might be the introduction of deep inspiratory (i.e., TLC) breaths that has the potential to decrease the effect of bronchoconstriction. This might explain the higher doses of histamine required to observe bronchoconstriction despite direct instillation into the lower airways.

5.2. Lobeline induced hyperpnea - alternative method for studying flow limitation

Injections of lobeline HCl (0.2 mg/kg) have been employed to cause hyperpnea in horses and thus simulate the conditions of exercise and peak inspiratory and expiratory flows [62,121,145,146]. Peak inspiratory and expiratory flows were 41±5 and 61±10 L/sec [146] in mature horses, respectively. Art [145] similarly found a greater effect to increase expiratory than inspiratory peak flows, exceeding flows obtained by light exercise and rebreathing. The peak expiratory flows caused by lobeline fall short of those seen during exercise, around 80 L/sec [128] but would appear to be sufficient for evaluation of expiratory flow limitation. In comparison, the forced expiration method [147] caused flow-limitation at near FRC at similar flow rates, although the large and prolonged negative pressure sink used for FEFV would be expected to induce greater airway compression. A direct comparison of methods is not available at the time of this writing.

The advantage of lobeline-induced hyperpnea over exercise is the avoidance of locomotory artifacts or effects of sympathetic stimulation characteristic of exercise, in addition to the shear simplicity of the lobeline injection. Lobeline injections (up to 3) can be repeated within a single day, and the effects on ventilation are highly reproducible [146]. One cited application of such repeated testing is the same-day evaluation of upper and lower airway function, using endoscopy and either auscultation or conventional mechanics, respectively. The current limitation is in the interpretation of results. One might hypothesize that horses with airway obstruction would show frequency dependence in Cdyn. There was no change in Cdyn or RL after lobeline bolus (0.2 mg/kg) in healthy horses [146], and a follow-up study showed that normal horses pre-constricted with methacholine, demonstrated frequency dependence [146]. Whether horses with naturally occurring mild or even severe airway obstruction demonstrate frequency dependence warrants further investigation. This largely depends on whether deep inspirations evoked by lobeline can overcome bronchoconstriction, a phenomenon that has not been resolved to date.

One technical problem with ventilatory measurements during lobeline is loss of linearity of the Fleisch pneumotachograph (No. 5) outside the specified range (0 - 25 L/sec), a problem that is not evident with ultrasonic pneumotachographs (e.g., Spiroson and BRDL pneumotachs) [146]. Kastner and coworkers [146] further derived a useful regression equation that allowed for correction of the flow rates in the non-linear range.

In conclusion, the full utility of lobeline-induced hyperpnea has not been completely explored, but there is great potential to understand dynamic lung function using this method.

6. Bronchoprovocation testing

6.1. Introduction to bronchoprovocation

Bronchoprovocation is a test that assesses the response of the respiratory system to bronchoconstrictor agonists. Airway reactivity is the expression of the overall dose vs. response of horses to these agonists. In sum, airway reactivity is a function of the threshold ("sensitivity") and magnitude ("responsiveness") of the response.
Airway hyper-reactivity (AHR) is by definition, an exaggerated narrowing response to a bronchoconstrictive stimulus, which was first found in horses with heaves [39]. The clinical correlates to AHR are thought to include coughing (increased sensitivity) and exercise intolerance (bronchoconstriction causing uneven ventilation and hypoxemia), and histologically, AHR correlates with airway wall thickening and infolding, mucus plugging, and increased inflammatory cells in and around the airway wall.

In ponies that respond to moldy hay challenge with airway obstruction, there is severe AHR [8,148] that can last several days to weeks [149]. Klein and Deegen [51] and Doucet and coworkers [150] described groups of horses with naturally occurring airway obstruction (cough, exercise intolerance, or heaves) that exhibited AHR without experimental exposure to moldy hay. Later it was showed that athletic horses with a history compatible with IAD exhibit significant AHR, and the magnitude of reactivity was surprisingly similar to heaves-susceptible horses [73,151]. Horses susceptible to moldy hay are at a higher risk of developing AHR [152-154]. Hence, the indoor environment is a major factor in the development or maintenance of AHR for susceptible horses, but the pathogenetic link remains elusive.

6.2. Theories behind the pathophysiology and pathogenesis of airway hyper-reactivity

Airway hyper-reactivity likely has several putative mechanisms, including excessive acetylcholine release, defective inhibitory non-adrenergic non-cholinergic (NANC) responses to stimulation, and lower than expected inhibitory function of prostanoids [152-154] in horses with heaves. With regard to inflammatory mediators, horses with IAD have increased leukotriene C4 in BAL fluid, in addition to higher %mast cells [73]. Mast cells may release leukotrienes, stimulating bronchoconstriction and mucus secretion. A study by Mazan [155] showed a lack of association between EIPH and IAD and between EIPH and AHR, therefore EIPH is not a consequence of AHR. Another study by Mazan [155] demonstrated higher AHR in rural than urban horses, suggesting that natural stimuli may be responsible for heightened airway reactivity in horses, supported by the aforementioned study by Vandenput [47] concerning the deleterious effects of stabling. In an earlier study by Hoffman [156], it was shown that influenza infection in ponies resulted in prolonged AHR, from 7 - 9 weeks after clinical recovery from influenza infection. There are many unanswered questions with regard to etiology, but most certainly feedstuffs include molds and endotoxin, and these are instrumental in exacerbating AHR in stabled horses.

6.3. Methods of bronchoprovocation

Bronchoprovocation tests are well suited for horses with suspected obstructive lung disease without clinical signs (i.e., heaves). It is inappropriate to test horses in exacerbation with heaves, as they will invariably possess AHR [47,51,148,150,151], and this will cause further distress.

Any of the lung function tests described in this chapter can be used for monitoring the effects of bronchoconstrictor agonists, i.e., during bronchoprovocation. The endpoints of course will vary depending on the device used for monitoring. For example, a 35% drop in Cdyn [148], a 35% increase in SFEmax by flowmetrics [121], or a 100% increase in R RS(1 Hz) using oscillometry [72,155] are evoked by similar concentrations of histamine. If one aims to find out the status of the airways prior to bronchoprovocation, this will require a sensitive test such as oscillometry of forced maneuvers at baseline. If airway reactivity is the only desired end-point for investigation, it is easiest to perform this test with oscillometry or flowmetrics, hence the latter two methods are employed for clinical or field testing in our hands. The methods for bronchoprovocation used in horses were adapted from those used in human [157]. After baseline lung mechanics are measured, saline (the solvent for histamine or methacholine) is nebulized as a control [75,148,155]. Following the test dose with the saline, a series of increasing doses of histamine di-phosphate (e.g., 1, 2, 4, 8, 16 and 32 mg/ml) or methacholine (0.001, 0.01, 0.1, 1.0, and 3 mg/ml) are administered, each followed by a measure of lung function. The test is discontinued when the measured response exceeds the desired endpoint. The lowest dose (1 mg/ml histamine) can be eliminated for outpatient testing since a response to this dose is highly unusual in non-heaves horses. A dose-response curve is generated (Fig. 14).

Figure 14. Shown is the method for interpolation of a dose-response curve. In this case, histamine challenge was monitored using oscillometry, but any method could be substituted. One may interpolate between the final two points that delineate the submaximal and supramaximal responses, or model the entire dose-response curve, using a semi-log dose - response curve for this purpose. The agonist must cause a change which is greater (supramaximal) or equal to the desired endpoint (e.g., 75% increase in resistance, 35% decrease of dynamic compliance) at which the interpolation is performed. - To view this image in full size go to the IVIS website at www.ivis.org.

The dose of histamine that evoked the pre-determined endpoint is interpolated on the dose-response curve. Newer systems (On the Nose®) analyze the dose-response curve and provide an index of airway reactivity, such as the dose of histamine that
increases respiratory system resistance by 75% ("PC75RRs"). The endpoint can be configured by the end-user. A low PC75RRs denotes greater airway reactivity. In general, normal reactivity is PC75Rss > 6 mg/ml, mild hyper-reactivity > 4 and < 6 mg/ml, moderate > 2 and < 4 mg/ml, and severe reactivity < 2 mg/ml. The index of airway reactivity should be re-measured after a suitable period of treatment, for example 30 days, to evaluate the response. A lack of change in airway reactivity denotes that either the horse has not received treatment or environment factors are overwhelming. We have also observed persistent AHR associated with high mast cell counts in BAL fluid, suggesting that AHR is linked to degranulation of these cells.

It is important to develop an algorithm that incorporates all the available clinical, lung function, and cytological data. One such algorithm, used in our clinic, is shown in (Fig. 15).

6.4. Clinical data in horses - normal, IAD, and heaves susceptible

Using oscillometry in normal horses with mean age of 7 yrs at Tufts University Large Animal Hospital, the author found AHR (PC75Rss < 6 mg/ml) in 27% (6 of 22) [151]. The mean value for this group of normal horses was 11 mg/ml histamine. The cutpoint was directly comparable [155] to the conventional cutpoint used by Klein and Deegen [51], i.e., the histamine dose that caused a 35% drop in Cdyn (PC35Cdyn). In the latter study, the reactive dose in normals was approximately 16 mg/ml histamine. In another study that examined normal stabled horses (n = 21) that were older (mean 14yrs), it was found that 18% showed severe airway reactivity defined as the concentration of histamine that increased SFEmax by 75% [75]. The mean value was 3 mg/ml. This denotes that underlying airway inflammation or airway wall thickening was prevalent in these older yet asymptomatic horses, and this condition goes unrecognized as a clinical entity due to their low-level athletic activity. In a recent study of young (2 - 4 year old) thoroughbred racehorses (n = 30) at Saratoga Racecourse (Saratoga Spring, NY, USA), normal (clinical, endoscopic, and performance histories) horses were tested using flowmetrics [158]. The reactive dose of histamine in these normal racehorses ranged 10 - 32 mg/ml. Hence, none of these normal performers exhibited AHR. These data are comparable to the normal horses in the Tufts Study [151] and the values of Klein and Deegen [51] in normal horses according to their respective definitions.

In horses with inflammatory airway disease/IAD (cough or exercise intolerance, and abnormal BAL cytology), we found by oscillometry that 40 out of 51 (78%) exhibited AHR (< 6 mg/mL histamine). All 40 horses with AHR had abnormal BAL cytology, and of the 11 horses without AHR, 9 had abnormal baseline lung function. Hence, 49 of 51 (96%) of horses classified as IAD based on clinical signs and BAL cytology, exhibited either AHR or abnormal lung mechanics. This indicates that lung mechanics should be combined with bronchoprovocation to define horses into such categories, and if baseline lung mechanics are not available, a BAL might be useful to increase the sensitivity for IAD.

In heaves-susceptible horses observed at the time of remission, 25 out of 30 (83%) reacted at PC75Rss < 6 mg/ml, and 29 out of 30 (96%) reacted at < 8 mg/ml. All 30 of these horses exhibited AHR, elevated baseline RRs, or elevated neutrophils (> 25%) in BAL samples. None of these horses were performing well, so they were presented for ongoing respiratory signs and queries concerning prognosis and how to manage them over the long term. These data exemplify the importance of integrating the baseline lung mechanics, bronchoprovocation, and BAL or other cytologic data to make these determinations, and to continue monitoring their progress.

Each laboratory must contrive a working definition of AHR, based on the method of testing and the population of horses tested. Young racehorses appear to have a different level of airway reactivity than older stabled horses. In our laboratory, we employ FOS and an endpoint of PC75Rss where a value of < 6 mg/ml histamine is defined as AHR [73,151]. Although it is compelling to perform a BAL in all cases, horses with AHR are expected to have abnormal BAL cytology, so the screening test may obviate the need for a BAL. Likewise, horses with normal baseline mechanics based on oscillometry that lack airway hyper-reactivity will not likely possess abnormal BAL cytology. Horses that are tested for airway hyper-reactivity alone and are negative, may have abnormal BAL but this occurs in less than 20% of the time. More stringent cut-points for AHR may improve sensitivity and specificity of lung function tests for IAD, but until a gold standard for IAD (other than BAL cytology and clinical history) are established, this will be open for debate.

It is recommended that horses get re-examined after a course of treatment with glucocorticoids, for example after 30 days of treatment. We do not request that the owners withdraw treatment of the horse, with the exception of phenylbutazone (72 hr), bronchodilators (48 hr), lasix (24 hr), and anti-histamines (7 days). Anti-histamines in particular will completely block the response to histamine, which seems obvious but many owners regularly give anti-histamines and need to be reminded to withdraw them.
This is the essence of a useful outpatient testing program that provides flexibility for clinicians to make decisions based on individual variations in owner concerns, economics, and the results of other tests.

7. Conclusion

In conclusion, lung function testing holds an important position in the science of equine respiratory diseases. The very basis of disease definition relies on the understanding of the functional properties of the animal. These properties will further allow us to define phenotype and as such, genotype and heritability in some cases (Fig. 16).

Figure 16. Lung function testing is at the center of development in our understanding of the pathogenesis, pathophysiology, pathology, epidemiology, diagnosis, and treatment of lung disease in the horse. With objective measures of lung function, it will be possible to describe new phenotypes and features of lung disease. Diagnostic tests may serve as the basis for a veterinary pulmonary specialty in the future. - To view this image in full size go to the IVIS website at www.ivis.org.

The objective measurement of lung function will be necessary in the investigation of disease incidence, prevalence, and risk factors. Since many horses have a more subclinical form of disease (IAD) that has the potential to reduce athletic ability, lung function testing will be a necessary addition to sports medicine evaluations, with the goal in mind to achieve the absolute best lung function in each horse. The tests described in this chapter are aimed at quantifying airway obstruction and airway reactivity. These will be particularly useful when coupled with other function tests that are not in widespread use, such as measurements of FRC, diffusion capacity, dead-space to tidal volume ratio, arterial-alveolar gas (O2, CO2) gradients, and ventilation-perfusion matching. Ultimately, the clinician with a special interest in respiratory medicine will be charged with the measurement and interpretation of a comprehensive array of tests in patients, at which point the specialty of veterinary pulmonology will be self-evident.

References


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