This is to certify that the

thesis entitled

Tidal breathing flow-volume loop analysis as a test of pulmonary function in the exercising horse

presented by

Bruce A. Connally

has been accepted towards fulfillment of the requirements for

MS degree in Large Animal Clinical Sciences

Major professor

Date 3/31/93

TIDAL BREATHING FLOW-VOLUME LOOP ANALYSIS AS A TEST OF PULMONARY FUNCTION IN THE EXERCISING HORSE

By

Bruce A. Connally

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Large Animal Clinical Sciences

1993

ABSTRACT

TIDAL BREATHING FLOW-VOLUME LOOP ANALYSIS AS A TEST OF PULMONARY FUNCTION IN THE EXERCISING HORSE

By

Bruce A. Connally

The usefulness of tidal breathing flow-volume loops to evaluate pulmonary function was investigated in six Standardbred horses during treadmill exercise. Tidal breathing flow-volume loops (TBFVL) are a graphical representation of airflow rate versus tidal volume for each individual breath. These TBFVL were obtained from horses exercising at speeds corresponding to 75% of maximum heart rate and maximum heart rate. Measurements were recorded in each horse before and after ovalbumin-induced allergic lung disease. Moderate obstructive lung disease, characterized by a significant increase in pulmonary resistance and a decrease in dynamic compliance, was observed while the horses were at rest. We found that in horses with airway obstruction exercising at 75% or 100% of maximum heart rate, the quantitative indices describing TBFVL shape and size were not markedly different from normal horses exercising at similar speeds.

I dedicate this thesis to Pat, Clint, and Matt.
It is their support and their love that has given me the strength
to make this change in my career.

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to Dr. Fred Derksen, my major advisor, for his guidance during the course of this study. I also wish to thank the members of my committee, Dr. Ed Robinson, Dr. Bob Bowker, and Dr. Judy Marteniuk, for their constructive criticism and editorial assistance in the preparation of this thesis. A special thanks is extended to Cathy Berney for technical assistance and to Vicki Kingsbury for thesis preparation.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

C_{dyn}	dynamic compliance
COPD	chronic obstructive pulmonary disease
EELV	end expiratory lung volume
$EF_{12.5}$	expiratory flow at 12.5% tidal volume
EF_{25}	expiratory flow at 25% tidal volume
EF_{50}	expiratory flow at 50% tidal volume
EIPH	exercise-induced pulmonary hemorrhage
f	respiratory frequency
FVC	forced vital capacity
HR	heart rate
HR_{max}	maximum heart rate
$IF_{12.5}$	inspiratory flow at 12.5% tidal volume
IF_{25}	inspiratory flow at 25% tidal volume
IF_{50}	inspiratory flow at 50% tidal volume
IVPF	isovolume pressure-flow curves
MEFV	maximum expiratory flow-volume
MEFVL	maximum expiratory flow-volume loops
MVV	maximal voluntary ventilation

PIF	peak inspiratory flow
R_L	pulmonary resistance
TBFVL	tidal breathing flow-volume loops
T_E	expiratory time
T_I	inspiratory time
V_E	expiratory volume
$\dot{V}_E$	minute ventilation
V_I	inspiratory volume
$\dot{V}O_{2max}$	maximal oxygen uptake
V_T	tidal volume
WR	work rate

INTRODUCTION

Respiratory abnormalities have been recognized as a common cause of poor performance in horses (Morris and Seeherman, 1991; Sweeney, 1991; MacNamara et al., 1990). Exercise-induced pulmonary hemorrhage (EIPH) and chronic obstructive pulmonary disease (COPD) are examples of two specific conditions that have been investigated and are characterized by airway obstruction (Sweeney, 1991; MacNamara et al., 1990; O'Callaghan et al., 1987e). Pulmonary function tests currently in use to evaluate airway caliber include the measurement of pulmonary resistance (R_L) and dynamic compliance (C_{dyn}) in the standing horse (Derksen et al., 1982a). Because the measurement of R_L and C_{dyn} is insensitive and only detects severe airway obstruction, the usefulness of these tests in the detection of potentially restricting lung disease is limited.

Maximum expiratory flow-volume loops (MEFVL) were first described by Hyatt et al. in 1958, and have proven to be the most sensitive test of pulmonary function in human medicine (Bass, 1973; Knudson et al., 1976). A subject must inhale to total lung capacity and subsequently exhale forcefully to residual volume to generate MEFVL. The technique is noninvasive and sensitive but requires patient cooperation to produce a maximal respiratory effort.

Tidal breathing flow-volume loops (TBFVL) have been used to evaluate airway caliber in dogs, horses, and human infants who were unable or unwilling to perform the forced vital capacity necessary to generate MEFVL (Amis and Kurpershoek, 1986; Petsche et al., in preparation; Godfrey et al., 1983). Because tidal breathing airflow and pressure changes in the airway are small, this technique is less sensitive in the detection of airway obstruction than the analysis of MEFVL.

I hypothesize that TBFVL obtained during exercise may be more sensitive in the detection of airway obstruction due to the greater airflow rates achieved with exercise (Belknap et al., 1989). The objective of this study was to evaluate TBFVL generated during exercise as a measure of pulmonary function in normal horses and in horses with induced airway obstruction.

CHAPTER I

LITERATURE REVIEW

The maximum expiratory flow-volume curve

Forced expiration to assess pulmonary function is common in human medicine. The maneuver is called a forced vital capacity (FVC). The patient is required to inspire maximally, then exhale as rapidly and completely as possible. Measurement of flow and volume of air during this maneuver can provide information about expiratory airflow limitation. The FVC maneuver is a useful measurement of pulmonary function because of the effort independence of maximal expiratory flow at all but near-maximal lung volumes (Hyatt et al., 1958). The relationship between pressure and flow at a given lung volume are shown in Figure 1 (Hyatt, 1986). The measurements were recorded with the subject in a body plethysmograph to record lung volumes.

The three curves at the right of Figure 1 are isovolume pressure-flow curves (IVPF) from a normal subject. The curve on the left shows a maximum expiratory flow-volume (MEFV) curve. This curve can be constructed by plotting the maximal flows from the IVPF curves against the lung volumes at which they are measured. The expiratory flow at point A is measured at a point of high lung inflation. This flow is directly related to increases in transpulmonary pressures. Thus expiratory flow near total lung capacity is dependent on the subject's effort.

At points B and C on the MEFV curve, the degree of lung inflation has decreased. The IVPF curves that correspond to these points show that flow increases with pressure until a maximal flow is reached. At that point further increases in pressure do not result in any significant increases in flow. Over the volume range corresponding to approximately the lower 80% of the vital capacity, the IVPF maxima do not require the subject to exert maximum effort but cannot be exceeded with maximum effort, resulting in measurements that are quite reproducible at these volumes. As can be seen, the maximum flow is a function of lung volume. As such it becomes essential to specify the lung volume in relation to maximum flow. A three-dimensional graphic representation of pressure, volume, and flow has been constructed by Fry and Hyatt (1960). The surface of this graph can be used to analyze the FVC but is difficult to construct and complex to interpret.

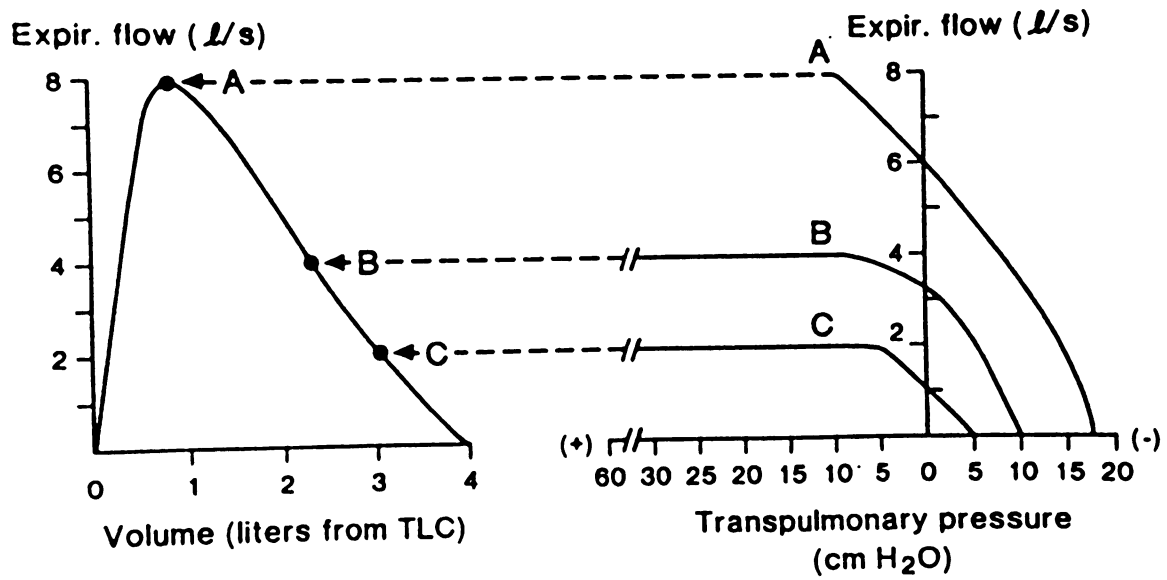

Figure 1. A maximum expiratory flow-volume (MEFV) curve from a normal subject (left). Values that compose this curve represent maximum flow at corresponding volumes. At right are three representative isovolume pressure-flow curves from the same subject. Curves A, B, and C were measured at the volumes represented by corresponding points on the MEFV curve. Transpulmonary pressure is the difference between pleural pressure and mouth pressure (from: Hyatt, 1986).

The equal pressure point

The mechanism of flow limitation during the MEFV maneuver has been studied extensively. The concept of an equal pressure point was introduced by Mead et al. in 1967. This theory stated that once flow was limited at a particular lung volume, there was an area in the intrathoracic airway where intrapleural pressure was equal to intrabronchial pressure. The elastic recoil pressure of the lung was the driving pressure from the alveolus to this equal pressure point. Airways downstream from this equal pressure point would be compressed because intrabronchial pressure would be less than intrapleural pressures, resulting in a narrowing of the airway.

This model of flow limitation is based on the principle of a Starling resistor. This device is a compressible tube passing through a chamber with adjustable pressure. Pressure differences between the input side of the collapsible tube and the chamber pressure determines flow, not the pressure drop between the input and output ends of the tube itself. This pressure-flow relationship is similar to the flow of water over a waterfall. Flow is determined by the pressure drop between the head of the river and the edge of the waterfall. The downstream segment can have no effect on the rate of flow. Pride et al. (1967) used this principle to describe flow limitation in the lung as a number of Starling resistors connected in parallel. If the critical transmural pressure applied over the collapsible airway is considered to be zero, this model is the same as the equal pressure point concept.

Wave speed limitation of flow

The analyses based on an equal pressure point or the Starling resistor concept does not completely explain flow limitation mechanisms. Dawson and Elliott (1977) concluded that the lung must obey the laws of fluid mechanics and thus cannot carry a greater flow than the flow for which the fluid velocity equals wave speed at some point in the system. This wave speed is the speed at which a disturbance travels in a compliant tube filled with fluid. The wave speed (c) in a compliant tube with an area (A) that depends on a lateral pressure (P), filled with a fluid of density (ρ), is given by:

$$c = \left(\frac{A\ dP}{\rho\ dA}\right)^{\frac{1}{2}}$$

where dP/dA is the slope of the pressure-area curve for the airway. Maximal flow ($\dot{V}_{max}$) is equal to fluid velocity at wave speed (c) multiplied by airway area (A). The resulting equation for maximal flow is:

$$\dot{V}_{max} = A\ \left(\frac{A\ dP}{\rho\ dA}\right)^{\frac{1}{2}}$$

The flow-limiting site or choke point occurs where the fluid velocity reaches the value of the local wave speed. Even this is not completely predictive because airway area,

compliance, and wave speed are functions of transmural pressures. Therefore the maximal flow that an airway can carry is a function of lateral pressure within the airway.

A more intuitive explanation of wave speed limitation has been provided by Hyatt (1986):

> Essentially one wishes to understand how the wave-speed mechanism produces plateaus on IVPF curves. The critical point to keep in mind is that on the plateau, flow is independent of the downstream pressure; this is the so-called "waterfall" or Starling resistor effect. In the isovolume condition at low flows, wave speed is high because the airways show little compression (area large) and are on the flat part of their pressure-area characteristic (they are stiff). A drop in pressure at the outlet will be transmitted upstream at a speed that is diminished only slightly by the countering effect of airflow velocity. As flow increases, the speed at which a downstream pressure disturbance (a drop in pressure) can be transmitted upstream will fall because 1) wave speed is decreasing and 2) the opposing effect of the airflow velocity is increasing. When the speed (predicted by the wave-speed equation) no longer exceeds the speed of expiratory flow, the pressure disturbance can no longer propagate upstream and affect the flow. In essence the downstream pressure change is no longer seen by the upstream driving pressure and flow becomes independent of, or uncoupled from, downstream pressure. When this occurs, the system is flow limited and the waterfall phenomenon and a

choke point develop. Further decreases in downstream pressure only compress the airways downstream of the choke point and, of course, have no effect on the flow.

The concept of flow limitation at wave speed is related to the equal pressure point concept described by Mead et al. (1967). The location of the choke point caused by wave speed limitation is anatomically very close to the equal pressure point (Dawson and Elliott, 1977). Dynamic airway collapse is a local phenomenon due to a pressure drop caused by wave speed limitation. The airway segment upstream from the choke point remains fixed during $\dot{V}_{max}$ and the cross sectional area of the airways remains constant. Downstream from the choke point there is a large pressure drop where energy is dissipated due to the inability of flow to increase.

Viscous limitation of flow

The wave speed flow limitation model is not as useful at low lung volumes (Dawson and Elliott, 1977). If airway diameters and flows are small, the limiting flow is essentially established by the coupling between flow limitations due to fluid viscosity and tube compliance before limitation at wave speed becomes significant.

Analysis of the flow-volume curve

Flow-volume curves are usually quantified by measuring $\dot{V}_{max}$ at various volumes in the vital capacity. Examples are 75%, 50%, and 25% of vital capacity remaining to be exhaled. These curves are not based on total lung capacity of the subject and are thus

termed free-floating curves (Hyatt et al., 1979). Because of the difficulty in measuring total lung capacity, the free-floating curves are most commonly used.

Defining normal values for MEFV curves has remained difficult. Variations due to age, sex, size, and race have been reported by Knudson et al. (1976). Intersubject variability may also reflect differences in mechanical properties of the lung. Green et al. (1974) reported that lung static recoil contributed little to the variability between individuals and concluded that the major variability in maximum flows was attributable to the airways. Green et al. (1974) argued that the most important airways are intrathoracic trachea and the large bronchi, and reported substantial between-individual differences in airway size and function that were independent of lung and parenchymal size. These differences may be due to disproportionate but physiologically normal growth within the organ. This phenomenon has been termed dysanaptic growth. It was also suggested that this variation in airway-parenchymal relationships between apparently healthy individuals might have an influence on the pathogenesis of airway disease.

When volume measurements are taken at the mouth, gas compression may also result in distortions that do not actually reflect lung volume changes. Ingram and Schilder (1966) have reported pressures of greater than 100 cm H_2O that may result in gas compression values of over 10%.

Another difficulty in interpretation is presented when repeated MEFV curves on the same subject result in moderate variation in VC, peak flows, and slope. Peslin et al. (1979) evaluated eight methods for comparing successive MEFV curves. They concluded that the most reproducible and unbiased data can be obtained from composite curves

obtained by superimposing the breaths either at total lung capacity or along the descending limb.

Clinical usefulness of the MEFV curve

Hyatt and Black (1973) state that the MEFV curve is a more valid and reliable way of studying expiratory flow events than is the spirogram, which plots flow against time during a FVC maneuver. The MEFV curve provides direct visualization of the reduction in $\dot{V}_{max}$ at all lung volumes, a value which is not as directly visualized on the volume-time plot of the spirograph. Hyatt and Black (1973) also describe reduction in $\dot{V}_{max}$ as the major mechanical expression of the obstructive process.

It is easy to inspect a series of MEFV curves to evaluate their general reproducibility. If there is doubt about a patient exerting sufficient effort to achieve $\dot{V}_{max}$, an esophageal balloon can be placed to determine if adequate pressures are being produced. If a subject is unable to produce a continuously forced expiratory effort due to lack of coordination or comprehension, an approximation of the MEFV curve may be attempted by superimposing repeated submaximal efforts at various levels of lung inflation (Hyatt and Black, 1973). Another means of defining MEFV curves in the noncompliant patient is to record a series of coughs from which the MEFV curve can be constructed (Hyatt and Black, 1973).

Maximum expiratory flow-volume curves have been used to evaluate patients with asthma (Bass, 1973; Chan-Yeung, 1973; Jordanoglou and Pride, 1968). Jordanoglou and Pride (1968) were able to differentiate asthma from severe fibrosis of the lung but were unable to distinguish asthma from bullous emphysema. Maximum expiratory flow at

50% of vital capacity was decreased in both asthma and bullous emphysema. Chan-Yeung (1973) also demonstrated a significant decrease in maximal expiratory flows at 50% of vital capacity and also at 60% of total lung capacity, even when patients were asymptomatic between asthmatic episodes. In three of nineteen subjects, asthmatic reactions were only detected by these measurements.

Bass (1973) was more successful in using MEFV curves to differentiate between clinical conditions. He reported patterns of abnormality for bronchial asthma as well as COPD, chronic bronchitis, and pulmonary emphysema. Flow limitation in large airways reduced the maximum expiratory flow at all lung volumes. Flow limitation in medium-sized airways decreased maximum expiratory flow rates primarily in the mid portion of the MEFV curve, while flow limitation in small airways reduced maximum expiratory flow at low lung volumes. Chronic bronchitis and bronchial asthma had similar reductions of flow in the mid portion of the MEFV curve. Differentiation of these two conditions was possible following isoproterenol administration. Only in patients with bronchial asthma did values revert toward normal. Pulmonary emphysema patients had more severe expiratory flow limitation than patients with either chronic bronchitis or bronchial asthma.

Hyatt and Black (1973) used MEFV curves to evaluate the effect of bronchodilator drugs. Maximum expiratory flow was compared at the same lung volume on curves recorded before and after therapy. If total lung capacity was measured, the curves were aligned in terms of absolute volume. If total lung capacity had not been measured, alignment was done at points of maximum inspiration. Response to therapy was readily visualized with this approach.

Maximum expiratory flow-volume curves during exercise

Attempts have been made to measure MEFV curves during or immediately following exercise in normal subjects (Olafsson and Hyatt, 1969; Stubbing et al., 1980) and in subjects with obstructive lung disease (Potter et al., 1971; Stubbing et al., 1980). Olafsson and Hyatt (1969) studied the interrelationships among transpulmonary pressure, flow, and volume during exhausting exercise. They then compared expiratory transpulmonary pressures during exercise with flow-limiting pressures measured at rest. No relationship was found between the magnitude of transpulmonary pressure and exercise limitation. Also, transpulmonary pressures in excess of the pressures necessary to achieve maximal airflow were not observed in these normal subjects.

Younes and Kivinen (1984) added support to the flow limitation concept by studying lung elastic recoil and inspiratory muscle fatigue at maximal exercise in normal subjects. They concluded that elastic recoil of the lung does not change with maximal exercise and that the measured decrease in C_{dyn} of the lung was not observed except at high levels of exercise. At near-maximal levels of exercise, these subjects' end-inspiratory volume was within 1.2 L of total lung capacity. Younes and Kivinen (1984) further state that this greater degree of lung inflation makes the lung much stiffer than it would be at a lesser lung volume.

Stubbing et al. (1980a) evaluated pulmonary mechanics during exercise by adapting a cycle ergonometer to a whole body plethysmograph. They were unable to document a change in MEFV curves of healthy subjects during exercise. The flow rates during tidal breathing at exercise did not exceed the MEFV curve. Like the findings of Olafsson and Hyatt (1969), even moderately severe exercise did not generate pressures

greater than those required to produce maximum flow. Stubbing et al. (1980a) also measured a decrease in static compliance, indicating a change in the elastic recoil properties of the lung. It was suggested that this reduction in static compliance may be due to the increase in pulmonary capillary blood volume during exercise, resulting in a less pliable lung.

Maximum expiratory flow-volume curves have also been recorded during (Stubbing et al., 1980b), and immediately after exhausting exercise (Potter et al., 1971) in subjects with airflow limitation. Potter et al. (1971) found that the expiratory pressure during exercise in patients with obstructive lung disease exceeded flow-limiting pressure measured at rest, and Stubbing et al. (1980b) found pressures to increase with exercise work load, but the MEFV curves were not altered significantly by these increased pressures.

Tidal breathing flow-volume loops

Some subjects, such as animals or human infants, cannot be induced to perform a forced vital capacity maneuver. In these subjects MEFV loops obviously are not applicable. Partial expiratory flow-volume maneuvers using various techniques have been performed in an attempt to quantify ventilatory mechanics in these subjects. Godfrey et al. (1983) utilized a technique for thoracic compression to generate partial expiratory flow-volume loops in human infants with lung disease. They compared the partial expiratory flow-volume loops with airway resistance and lung volume measurements obtained from whole body plethysmography, and the effects of inhaling a helium/oxygen gas mixture to partition the airway obstruction between large and small

airways. In several instances they were able to detect small airway obstruction without changes in airway resistance or total lung capacity.

Another technique for obtaining flow-volume loops in infants was utilized by Wise et al. (1980). A crying vital capacity maneuver was performed by "gently stimulating the infant to cry into a snugly-fitting rubber-rimmed face mask." Infants with hyaline membrane disease had reduced "crying vital capacity" when compared to normal infants. The authors suggested that the low expiratory flows may occur due to 1) loss of lung volume and diminished recoil pressures; 2) increased resistance at the vocal cords due to vocal tone during crying; and 3) partial collapse of major intrathoracic airways due to elevated pressures developed during crying.

Tidal breathing flow-volume loops analysis has been performed on dogs (Amis and Kurpershoek, 1986) and horses (Petsche et al., in preparation) at rest to evaluate airway obstruction. Because these TBFVL are effort dependent, absolute values for flow and volume as well as breathing pattern used are difficult to standardize. Abnormalities of TBFVL associated with flow limitation might not become apparent until pulmonary flow reserves are dramatically reduced (Amis and Kurpershoek, 1986). In this case the animal may be approaching the MEFV curve during tidal breathing.

Amis and Kurpershoek (1986) reported that absolute values for flow and volume in dogs were affected greatly by body size and amount of effort. They concentrated on the shape of the TBFVL to eliminate body size differences. The measurements proved to be insensitive due to the large variability in results obtained.

Petsche et al. (in preparation) used TBFVL to examine horses with recurrent airway obstruction (heaves). Horses with severe airway obstruction, as demonstrated by

increase in R_L and decrease in C_{dyn} of the lung, had increased peak expiratory flow early in expiration compared to normal horses. Horses with obstructive lung disease had TBFVL that differed from normal TBFVL by the absence of a biphasic pattern on inspiration or expiration, even when the obstruction did not result in an increase in R_L or decrease in C_{dyn}.

Tidal breathing flow-volume loops have also been measured during exercise in humans and animals. Stubbing et al. (1980a) measured TBFVL during exercise in normal adult males and compared the results to MEFV curves obtained when the subject had reached a steady state at each work load increment. The tidal breathing expiratory flow rates measured during exercise did not exceed the MEFV curve. In two of twelve subjects the flow during tidal breathing at the highest work load reached the MEFV curve.

Art and Lekeux (1988) studied exercise-induced changes in equine breathing pattern by analyzing TBFVL in ponies at rest and during a standardized exercise. They found that rest and exercise TBFVL varied between ponies but remained relatively constant for a given animal. Ratios relating peak expiratory flow to peak inspiratory flow, and expiratory flow at 50% and 25% of tidal volume to inspiratory flow at the same intervals were significantly increased. The other indices did not change significantly except for peak expiratory flow/expiratory flow at 50% of tidal volume, which was decreased.

Lumsden et al. compared the TBFVL at rest and during strenuous exercise for evaluation of upper airway obstruction in the Standardbred horse. They found significant

inspiratory flow limitation during exercise using TBFVL. The same loops were found to produce no useful information when the horses were at rest.

The use of TBFVL during exercise to evaluate obstructive lung disease has been more limited than the use of MEFV curves. Potter et al. (1971) compared TBFVL measured while individuals were exercising on a treadmill to MEFV curves measured before exercise. In normal subjects the exercise TBFVL rarely impinged on maximum inspiratory or expiratory flow-volume curves except at low volumes. Patients with obstructive lung disease reached maximal expiratory flow predicted from their MEFV curves before heart rate (HR) was maximal, prompting the authors to conclude that exercise capacity was limited by ventilation.

Stubbing et al. (1980b) also compared TBFVL during exercise to MEFV curves in patients with chronic airflow obstruction. They had the patients exercise within a whole body plethysmograph to facilitate measurement of vital capacity, MEFV curves and TBFVL at each exercise increment. The MEFV curves did not change as a result of exercise, and tidal breathing flows equalled but did not exceed the maximal flows at similar lung volumes.

Pollmann and Hörnicke (1987) evaluated TBFVL during exercise in horses with reduced performance due to pulmonary emphysema or bronchitis. Tidal volume and inspiratory flow rates were increased in horses with obstructive lung disease compared to healthy horses. The authors concluded that respiratory airflow of horses with COPD differs from that described for human subjects with obstructive pulmonary disease.

It is possible that the increased flow rates found by Pollmann and Hörnicke (1987) may not be physiologic, but may be a function of the placement of the TBFVL on the

volume axis. Potter et al. (1971) and Stubbing et al. (1980b) both were able to report an increase in end expiratory lung volume (EELV) in their patients during exercise. Stubbing et al. (1980b) states:

> The MEFV curve defines the limits of flow at a given lung volume and subjects who breathe along the same FV curve at rest as during maximal expiratory efforts should only be able to increase their total ventilation by either increasing inspiratory flow, allowing more time for expiration, or by increasing the end-expiratory volume, allowing greater expiratory flow.

The regulation of EELV during exercise has been evaluated in healthy subjects by Henke et al. (1988). They were able to demonstrate a reduction in EELV with increasing exercise intensity. This reduced EELV during exercise was thought to aid inspiration by optimizing diaphragmatic length and permitting elastic recoil of the chest wall. Babb et al. (1991) also demonstrated this exercise-associated reduction in EELV in healthy subjects but found that patients with mild-to-moderate airflow limitation responded quite differently. As predicted by Stubbing et al. (1980b), Babb et al. (1991) found that patients with airflow limitation actually increased EELV to 58% of total lung capacity, whereas controls decreased EELV to 45% of total lung capacity. They concluded that the increase in EELV was due to the effects of airflow limitation on the ventilatory response to exercise. By moving the TBFVL along the volume axis to utilize higher lung volumes, greater flows could be achieved before being limited by the MEFV

curve. It is possible that this adjustment of EELV by a flow-limited patient could account for the increased flows reported by Pollmann and Hörnicke (1987) in horses with COPD.

Clinical exercise testing

Exercise testing is currently used in human athletes for fitness evaluation. It also enables simultaneous evaluation of the cardiovascular and respiratory systems. The severity of a disease and its response to therapy can be monitored by measuring aerobic capacity, anaerobic threshold, ventilatory response, and hypoxemia. The scope of this review is, of necessity, limited to the ventilatory aspects of clinical exercise testing.

The exercise testing protocol may be performed by incremental increases in work load to reach a maximum work rate (WR) in a relatively short period of time or by maintaining a constant WR for a prolonged time period. The constant WR allows the investigator to measure physiological responses of specific organ systems to the stresses of exercise but is not as easily applied to the clinical setting. Wassermam et al. (1987) states three main reasons for the use of the incremental exercise testing protocol. First, it does not require a sudden large cardio-respiratory stress because the testing begins at relatively low WRs. Second, the test requires a relatively short period of time during which the subject is stressed at high WRs. Finally, a relationship may be determined or estimated between maximal oxygen uptake and WR for each subject.

Equipment used may be as simple as a stairway to provide exercise and equipment for data collection during intermittent stops. A treadmill or cycle ergonometer provides a more controlled exercise stress and allows continuous monitoring of data. The severity

of the exercise stress is increased incrementally until the patient reaches his/her aerobic capacity.

Many different parameters may be measured to evaluate maximal oxygen uptake ($\dot{V}O_{2max}$), anaerobic threshold, and ventilatory response to incremental exercise. The electrocardiogram (ECG) can be used to monitor the myocardial oxygen availability-requirement balance.

At any level of training the body has an upper limit for oxygen utilization, which is determined by cardiac output, oxygen extraction by the tissues, or by having reached a ventilatory limit. Wasserman et al. (1987) state that $\dot{V}O_{2max}$ can be determined by incremental exercise testing when a plateau occurs in oxygen uptake despite further increases in WR. Thus $\dot{V}O_{2max}$ is a reproducible measurement of aerobic capacity, which is defined by Weber et al. (1988) as a change in oxygen uptake of < 1 ml/min/kg sustained for a minimum of thirty seconds into the next stage of incremental treadmill work, but preferably for a full stage or two minutes. By plotting oxygen uptake against external work performed, information concerning the coupling of internal and external respiration can be ascertained.

The anaerobic threshold is defined by Wasserman et al. (1987) as the level of exercise $\dot{V}O_2$ above which aerobic energy production is not adequate and is supplemented by anaerobic mechanisms. The result is an increased blood lactate and lactate/pyruvate ratio. This can also be monitored by measuring the rate of increase in $\dot{V}CO_2$ relative to VO_2 during the incremental exercise test. Weber et al. (1988) have suggested use of the anaerobic threshhold as a means of evaluating whether patients can be encouraged to reach additional increments of exercise stress to achieve $\dot{V}O_{2max}$.

The ventilatory response to incremental exercise testing can also be measured and is of primary interest to this author. Oxygen delivery during exercise cannot be maintained without increases in cardiac output and minute ventilation ($\dot{V}_E$). During aerobic work $\dot{V}_E$ rises in proportion to both $\dot{V}O_2$ and $\dot{V}CO_2$. During anaerobic work buffering of the lactic acid produces more CO_2, which must be removed by ventilation.

Weber et al. (1988) has stated that in normal human subjects the maximal $\dot{V}_E$ at $\dot{V}O_{2max}$ is less than fifty percent of the maximal voluntary ventilation (MVV). The MVV is usually measured over a 12- or 15-second time period and is motivation and effort dependent. The difference between the MVV and maximum $\dot{V}_E$ during exercise is used as a measure of the ventilatory or breathing reserve (Weber et al., 1988). The increase in $\dot{V}_E$ is accomplished by increases in tidal volume and breathing frequency.

With the advent of high-speed treadmills, clinical exercise testing has recently become a practical technique for use in horses. While this technique has not yet been utilized to determine fitness of athletes, studies have been undertaken to establish normal values of some physiological parameters (Evans and Rose, 1983; Evans and Rose, 1988; Rose et al., 1990).

The exercise testing used a protocol of incrementally increased work load performed on a six degree-inclined motorized treadmill. Oxygen uptake ($\dot{V}O_2$), HR, $\dot{V}_E$, and cardiac output were measured. Work rate was calculated using the formula:

$$\text{WR (watts)} = \frac{\text{velocity (m/min)} \times \text{body weight (kg)} \times \sin \text{(treadmill angle)}}{6.12}$$

Evans and Rose (1983, 1988) reported linear relationships between $\dot{V}O_2$ and WR, HR, $\dot{V}_E$, and cardiac output in detrained Standardbred and Thoroughbred horses. The $\dot{V}O_{2max}$ increased significantly with training. There was a linear relationship between the percentage of $\dot{V}O_{2max}$ and the percentage of HR_{max} but no relationship between $\dot{V}O_{2max}$ and HR_{max} was established.

Lumsden et al. utilized a variation of this clinical exercise testing protocol to estimate the relationship between HR and treadmill speed for individual horses. This rapid incremental exercise test (RIET) was performed on a three-degree-inclined treadmill. Speed was increased at sixty-second intervals and HR was recorded in the last 15 seconds of each exercise period. Maximum HR and calculated 75% of HR_{max} for each individual horse were then used to calculate a treadmill speed for each animal that produced a standardized WR to evaluate TBFVLs.

The continued development of more sensitive techniques to evaluate respiratory function in the horse is necessary. Bayly and Slocombe (1991) state that poor athletic performance in horses is often linked to abnormal respiratory function. Many affected animals are asymptomatic at rest. Because of this, accurate clinical evaluation becomes impossible unless some form of clinical exercise testing is utilized. Evaluation of changes in C_{dyn}, functional residual capacity, oxygen utilization, and small airway function at exercise would greatly improve the assessment of the pulmonary function in the equine athlete. Concerning the development of this clinical exercise testing, Bayly and Slocombe (1991) have written that:

Such developments are essential if the pathogenesis of specific entities such as exercise-induced pulmonary hemorrhage, and non-specific subclinical problems like bronchiolitis, are to be better understood.

My studies, reported in this thesis, are aimed at the development of clinically useful tests of pulmonary function. In the next chapter I will report on exercise testing of normal horses and horses with ovalbumin-induced allergic lung disease. All of the horses with induced airway obstruction developed severe exercise-induced pulmonary hemorrhage. Because of this, and because EIPH is such a common occurrence in exercising horses, a review of EIPH follows.

Exercise-induced pulmonary hemorrhage

Epistaxis after exercise has been observed in horses for many years. It has commonly been referred to as "bleeding," "broken blood vessels," or "epistaxis" (Pascoe et al., 1981). The origin of this hemorrhage was generally considered to be the nasal cavity until Mahaffey (1962) suggested a pulmonary source based on necropsy examinations. Since that time, endoscopy has been used to support the theory that the hemorrhage originates in the caudal part of the lung (Pascoe et al., 1981; Hillidge et al., 1984, 1985; Raphel and Soma, 1982). On the basis of these observations Pascoe et al. (1981) suggested the term "exercise-induced pulmonary hemorrhage" to more accurately describe the clinical condition in the racing horse.

The frequency of EIPH based on the appearance of blood at the nostril after exercise is quite low, but it has been reported to be much higher when diagnosed by

endoscopy. In Thoroughbreds EIPH prevalence has been reported to be between 44 percent and 75 percent (Pascoe et al., 1981; Raphel and Soma, 1982). Hillidge et al. (1984, 1985) have reported similar rates in other racing breeds.

Association of EIPH with the age of the horse has been shown by Hillidge et al. (1984, 1985) and Raphel and Soma (1982). They were able to show that frequency of EIPH increased with increased age of the horses.

The distance a horse is raced has also been related to EIPH. Raphel and Soma(1982) were able to show a direct relationship between distance raced or breezed and EIPH in Thoroughbreds. This association was not detected in racing Appaloosa or Quarter horses, but Hillidge et al. (1984, 1985) suggested that it was the intensity of exercise, rather than the distance run, which affected the prevalence of EIPH in these breeds.

The effect of EIPH on performance remains controversial. In the Thoroughbred horse a relationship between EIPH and finishing position was not observed by Pascoe et al. (1981) or Raphel and Soma (1982). However, when endoscopic examinations were performed by MacNamara et al. (1990) on Standardbred racehorses, the findings suggested that both EIPH and COPD adversely affected performance. A combination of the two conditions more severely affected racing performance than did either condition by itself. Raphel and Soma (1982) were unable to relate the degree of hemorrhage to finishing position. Historically horses that bled from their nostrils have been considered more severely affected, but Raphel and Soma (1982) suggest that absence of blood at the nostril may not reflect severity of hemorrhage observed by endoscopic examination.

Clinical signs of EIPH in affected horses may range from blood flow out of both nostrils to no detectable external abnormalities. Frequent swallowing after exercise may indicate that the horse is swallowing blood that is flowing into the pharynx from the trachea (Pascoe et al., 1981). O'Callaghan et al. (1987a) have reported that focal abnormal lung sounds may be auscultated in the dorsocaudal lungfields during rebreathing, but the findings are inconsistent.

Radiographic changes associated with EIPH have been documented (Pascoe et al., 1987; O'Callaghan et al., 1987b). Abnormal pulmonary opacities were identified in the caudal lung lobe. These opacities were large and peripherally located, obliterating the thoracophrenic angle, but often did not have sharp margins. The intensity of the opacification of the consolidated area varied. Pleural effusion was detected in nine of thirteen horses. Pascoe et al. (1987) found that these pulmonary radiopacities cleared in ten days in two horses but required months to resolve in five others. O'Callaghan et al. (1987b) coded estimates of radiographic lesion opacity and attempted to correlate this with hemosiderin deposits and bronchial artery neovascularization. They concluded that accurate prediction of lung lesions based on radiographic criteria was difficult.

Pathologic changes

The gross lesions associated with EIPH are seen in the dorsocaudal regions of the lung and in some cases areas of discoloration have been observed over as much as one-third of the lung surface (O'Callaghan et al., 1987c). The lesions are characteristically stained a light brown-bronze color and in the inflated lung have a fine reticular pattern. These discolored areas of lung are denser than normal, collapse less readily, are slower

to inflate, and may contain pockets of trapped gas. The subpleural bronchial arteries are more prominent and appear to be enlarged.

Histopathologic examination of the affected regions of lung shows extensive hemosiderin deposition. Microangiographs of sections of lungs that were perfused with latex reveal proliferation of the bronchial arterial circulation in regions with evidence of pulmonary hemorrhage (O'Callaghan et al., 1987d). There was also evidence of small airway disease. The most severely affected bronchioles contained mucous exudate or mucoid plugs and had grossly thickened walls characteristic of bronchiolitis (O'Callaghan et al., 1987e). It was suggested that the small airway disease may have precipitated the bronchial arterial proliferation. Microradiography and computerized tomography scanning were used to identify plexuses of markedly hypertrophied bronchial arterial networks centered around small airways (O'Callaghan et al., 1987f). The authors concluded that the bronchial vascular lesions are the likely source of hemorrhage in EIPH.

O'Callaghan et al. (1987g) used ventilation/perfusion scintigraphy to demonstrate pulmonary arterial perfusion impairment. Ventilation deficits in the same areas also suggested that there was small airway disease as noted on histopathology.

Pathogenesis

The pathogenesis of EIPH is unknown at this time although many theories have been presented. As it became known that the origin of bleeding was the lung instead of the upper airway, emphasis has been on an underlying pulmonary disease that could

predispose the lung to injury during exercise (MacNamara et al., 1990; O'Callaghan et al., 1987h; Pascoe et al., 1989; Robinson and Derksen, 1981).

Robinson and Derksen (1981) proposed that small airway obstruction may be a cause of EIPH. This small airway obstruction would result in asynchronous ventilation of pulmonary lobules. Because all structures of the lung are interconnected, movement of one area will exert forces on adjacent areas. The areas of lung that inflate normally then exert expansive forces on uninflated areas. This interdependence of lung structures may create local areas of increased distending forces in the resting horse's lung. If airway obstruction is incomplete, these additional forces may be able to fully inflate the affected areas but the time required for inflation will be prolonged. While this delayed filling may not be sufficient to cause signs of impaired gas exchange at rest, during exercise the partial obstruction may prevent filling of the alveoli distal to the obstruction in the time available for inhalation. Thus even a slightly obstructed segment moves asynchronously with the adjacent lung tissue. This asynchrony precipitates an accentuation of distending forces that may result in tearing of lung parenchyma with concomitant pulmonary hemorrhage. Inelastic scars within the lung or pleural adhesions that do not expand with lung volume may be contributing factors. A second effect that may occur with asynchronous ventilation of the lung is the transmission of the additional distending forces into the obstructed alveolus. Intraalveolar pressures would decrease more than normal during a respiratory cycle. This accentuated decrease in alveolar pressure and presumed increase in pulmonary capillary pressure during exercise could elevate capillary transmural pressure. If this increase in capillary transmural pressure is great enough, rupture of capillary endothelium and intraalveolar hemorrhage would

result. If airway obstruction is incomplete then the hemorrhage has a route to the trachea.

The dorsocaudal distribution of lesions found in EIPH has been attributed to regional differences in pulmonary mechanics. Milic-Emili et al. (1966) proposed that a vertical pleural pressure gradient would result in alveoli being more distended in the dorsal regions of the lungs. Robinson and Derksen (1981) proposed that during rapid breathing the narrower airways in the base of the lung may delay filling of that region. Thus, the dorsal region of the lung is more susceptible to excessive distension, which may result in tearing of lung parenchyma and subsequent EIPH.

A more recent study by Derksen et al. (1992) used an ovalbumin-induced allergic lung disease model to create bronchiolitis and hemorrhage in the lung. This hemorrhage did not appear in the large airways unless the horses were exercised on a treadmill. The mechanism that caused the hemorrhage to appear in the large airways is unknown. Derksen et al. (1992) postulated that vascular pressure increases or mechanical forces exerted on the lung during exercise may have caused an increased amount of hemorrhage. It is also possible that strenuous exercise served only to mechanically move blood from the alveoli to the larger airways. In this case, the term "exercise-induced pulmonary hemorrhage" would be inaccurate, as hemorrhagic lung lesions may be present prior to exercise.

Derksen et al. (1992) also used the ovalbumin-induced allergic lung disease model to examine the importance of regional differences in interdependence forces in the lung to the pathogenesis of EIPH. Blood appeared in the large airways following exercise after ovalbumin-induced lung disease in the dorsal, middle, and apical regions of the

lung. These findings do not support regional differences in interdependence forces as the explanation for the dorsocaudal distribution of lesions found in EIPH.

Another theory of the pathogenesis of EIPH proposed by O'Callaghan et al. (1987h) is predicated on the presence of preexisting small airway disease. In this study the primary lesions were multifocal small airway disease in the dorsocaudal lungfields and "an intense vascular proliferation resulting in apparent domination of the alveolar vascular bed by the bronchial arteries." Other changes found on histopathology such as hemosiderophage sequestration, fibrosis, and lung parenchyma destruction were thought to be secondary to previous incidents of hemorrhage and inflammation.

The proliferation of bronchial vasculature is proposed to occur as part of the normal inflammatory repair response to focal areas of bronchiolitis. If the horse is exercised during this inflammatory response, a localized hypoxia is produced in the affected secondary lobules. This stimulates a bronchial arterial angiogenesis, which the authors suggest may be mediated by activated macrophages. In these areas of hypoxia there is a reflex reduction of local pulmonary arterial flow in order to redistribute blood flow away from non-ventilated areas and thus maintain a balanced ventilation/perfusion ratio throughout the lung. Flow to the alveolar capillary bed is then maintained from bronchial arteries through precapillary anastomoses. The resultant bronchial arterial proliferation potentially may result in increased anastomotic flow and higher pressures at the arteriolar-capillary junction of the alveoli. Based on this pathogenetic theorem, hemorrhage in EIPH is probably of bronchial arterial origin.

Although an etiologic agent was not identified, O'Callaghan et al. (1987h) speculated that the most likely cause may be a respiratory virus. Rhinopneumonitis

(EHV-1) virus was suggested as a possibility because of its high morbidity, low mortality, worldwide distribution and the potential for horses to be infected repeatedly.

Exercise-induced pulmonary hemorrhage does not exclusively involve airways, but an interaction between the respiratory and circulatory systems. Indeed the most commonly used treatment for EIPH, furosemide, reduces plasma volume (Freestone et al., 1988), right atrial pressure, pulmonary arterial pressure (Goetz and Manohar, 1986; Olsen et al., 1992), and mean aortic pressure (Erickson et al., 1992).

Erickson et al. (1989) found pulmonary artery pressure increases to more than 80 mm Hg in the exercising horse. The reasons for this marked pulmonary hypertension are not known. Goetz and Manohar (1986) suggest that the increase in pulmonary artery pressure of exercising ponies may be due to a loss of compliance in the pulmonary arterial system. Another possibility is that an increase in packed cell volume caused by splenic contraction results in hyperviscosity of the blood (Davis and Manohar, 1988; McClay et al., 1992). This hyperviscosity was ameliorated by splenectomy in ponies (Davis and Manohar, 1988). Cardiac output was unchanged, but systemic and pulmonary arterial pressures were significantly decreased. Total pulmonary vascular resistance and total peripheral resistance were also significantly decreased, suggesting that an increase in packed cell volume may contribute substantially to pulmonary and systemic hypertension of exercise in ponies. A considerable decrease in the exercise capacity of the splenectomized ponies was also shown.

Packed cell volume increases of 58 to 61% and blood viscosity increases of 2 to 3 times were reported in racing Thoroughbreds by McClay et al. (1992). It was suggested that postrace changes of this magnitude may impede blood flow and may result

in microvascular injury. The authors, however, were unable to differentiate between horses affected with EIPH and normal horses using changes in hematologic and rheologic parameters. They postulated that all horses may be predisposed to EIPH and clinical manifestations of bleeding may be dependent on factors such as duration of racing career, effort expended in a race, and the number of postrace bronchoscopic examinations.

The high pulmonary vascular pressures in the exercising horse could potentially be damaging to pulmonary capillaries, but this has not been measured directly. Manohar (1992) has calculated pulmonary capillary pressures by averaging measured pulmonary artery pressure and pulmonary arterial wedge pressure in the exercising horse. In horses running on a treadmill at 14 m/s the calculated pulmonary capillary pressure was 79 mm Hg. At capillary pressures greater than 40 mm Hg, West et al. (1991) was able to demonstrate disruption of the capillary endothelium and alveolar epithelium in rabbits. This disruption of pulmonary capillaries was termed stress failure and has also been reported by West et al. (1992) in two racehorses. The two horses were known bleeders and were galloped at maximum speed on a treadmill before being euthanatized.

Treatment

Many therapeutic regimens have been proposed to prevent EIPH in performance horses. Management changes, furosemide, bronchodilators, hesperidin and citrus bioflavonoid, estrogens, and water vapor therapy have been recommended (Sweeney, 1991). Of these furosemide is probably the most widely used. Furosemide appears to have limited effectiveness for the prevention of EIPH but may be able to diminish the amount of hemorrhage during a race and may improve performance to pre-EIPH levels

(Erickson et al., 1989; Pascoe et at., 1985; Sweeney et al., 1990). Furosemide has also been shown by Sweeney et al. (1990) to enhance performance in geldings but not in mares and stallions without a diagnosis of EIPH.

The mechanism of action by which furosemide alters performance is unknown. Freestone et al. (1988) state that furosemide does not decrease PCV, plasma sodium concentration, or osmolality. Geor et al. (1992) associated furosemide with increases in mean RBC hemoglobin concentration and blood viscosity. These factors could exacerbate exercise-associated hyperviscosity in horses. These findings do not correlate with reported decrease in EIPH after administration of furosemide (Erickson et al., 1989; Pascoe et al., 1985).

Furosemide has been shown to decrease right atrial and pulmonary arterial pressures (Goetz and Manohar, 1986; Olsen et al., 1992). The significance of this decrease is questionable. In one study, mean pulmonary artery pressure in the exercising horse was still 240% of the resting value (Goetz and Manohar, 1986). Olsen et al. (1992) reported that this reduction in pulmonary artery and right atrial pressures can be partially reversed by administration of the cyclooxygenase inhibitors phenylbutazone and flunixin meglumine. This suggests that furosemide may mediate its vascular effects by influencing arachadonic acid metabolism.

Erickson et al. (1992) did not demonstrate an effect of furosemide on pulmonary artery pressure but did show a reduction in mean aortic pressure during exercise. The lack of effect on pulmonary artery pressure was explained by time of furosemide administration before exercise. In this study one hour elapsed between furosemide administration and exercise. In other studies where furosemide affected pulmonary

arterial pressures but not aortic pressures, the drug was administered 2 to 4 hours before exercise (Goetz and Manohar, 1986; Olsen et al., 1992). Olsen et al. (1992) suggests that the regulation of vascular tone by arachadonic acid metabolites may differ between the pulmonary and systemic circulations.

Furosemide has also been shown to have an effect on airways. Exercise-induced bronchoconstriction in humans was prevented by treatment with inhaled furosemide (Bianco et al., 1988). Broadstone et al. (1970) demonstrated that inhaled furosemide attenuates airway obstruction in ponies with recurrent obstructive pulmonary disease. It is possible that the effects of furosemide on airways may be important in the dimunition of hemorrhage during a race and the improvement of performance to pre-EIPH levels reported by Sweeney et al. (1990).

Conclusion

Exercise-induced pulmonary hemorrhage is a complex and still poorly understood syndrome. The clinical signs and lesions have been described in detail but pathogenesis remains unclear. Because this syndrome only occurs during strenuous exercise, clinical exercise testing is an indispensable tool in the elucidation of EIPH. Because O'Callaghan et al. (1987e) reported small airway disease in horses with EIPH, a sensitive and non-invasive means of evaluating pulmonary function in the exercising horse would appear to be mandatory to the understanding of this complex entity.

In this study we evaluated TBVFLs obtained during exercise as a measure of pulmonary function in normal horses and in horses with ovalbumin-induced airway obstruction. Derksen et al. (1992) used ovalbumin to induce allergic lung disease

characterized by bronchiolitis and EIPH. Therefore, this model was used during my thesis research.

CHAPTER II

TIDAL BREATHING FLOW-VOLUME LOOP ANALYSIS AS A TEST OF PULMONARY FUNCTION IN THE EXERCISING HORSE

Materials and methods

Six Standardbred horses between 3 and 11 (mean 5.17 $\pm$ 1.33 S.E.M) years of age were used in this experiment. The horses were immunized against equine influenza and rhinopneumonitis virus and were on pasture for at least one month before use in the experiments. A physical examination, including auscultation of the heart and lungs, and endoscopic examination of the upper airway at rest and while exercising on the treadmill revealed no abnormalities. Experimental procedures were reviewed and approved by Michigan State University's All-University Committee on Animal Use and Care.

Sensitization of horses

Horses were sensitized to ovalbumin by intramuscular injection using 10 mg of ovalbumin dissolved in 1.5 ml of phosphate-buffered saline and emulsified in 1.5 ml of complete Freund's adjuvant. This emulsion was divided and injected deep into the left and right semimembranosus muscles. Two weeks later the protocol was repeated using incomplete instead of complete Freund's adjuvant.

Induction of disease

At least two weeks after the last injection, the horses were restrained in stocks and fitted with a mask and non-rebreathing valve. An ultrasonic nebulizer (Model 65 ultrasonic nebulizer, De Vilbiss, Somerset, PA) was used to administer an aerosol of 20 ml phosphate-buffered saline containing 1 gram ovalbumin to each horse over a 15-minute period.

Pulmonary function measurements

Experiments were performed with the horses restrained in stocks without sedation. Pulmonary function was measured as previously described by Derksen et al.(1982a). Pleural pressure was measured using an esophageal balloon (10 cm long, 3.5 cm perimeter, 0.06 cm wall thickness) sealed over the end of a polypropylene catheter. The balloon was passed through the nares into the mid-thoracic portion of the esophagus and connected to a pressure transducer (Validyne Model DP/45-35, Northridge, CA). This pressure transducer was calibrated before each study using a water manometer. A face mask was placed over the muzzle and a rubber shroud and tape were used to seal the mask to the face. A pneumotachograph (No. 5 Fleisch, Grand Rapids, MI) attached to the mask was connected to a pressure transducer (Validyne DP/45-22, Northridge, CA) that produced a signal proportional to airflow. This signal was processed by a lung function computer (Model LS-14, Buxco Electronics, Inc., Sharon ,CT), which integrated the signal to give tidal volume. The pneumotachograph transducer system was calibrated using a 2-liter syringe. Flow, tidal volume, and pleural pressure were used by the computer to calculate R_L, C_{dyn}, respiratory frequency (*f*), and $\dot{V}_E$.

Rapid incremental exercise test

Maximum HR during exercise was established prior to airway function tests. A rapid incremental exercise test was performed to estimate the relationship between HR and treadmill speed for each horse. This exercise test was similar to that reported by Rose et al. (1990) except that a 3-degree inclined treadmill was used. Horses were exercised at 4 m/sec for 3 minutes, then at 6 m/sec for 90 seconds. Treadmill speed was then increased at one-minute intervals to 8, 10, 11, and 12 m/sec. The test was stopped when the horse could no longer maintain position on the treadmill. Heart rate was recorded in the last 15 seconds of each exercise period. The speed at which the horse reached HR_{max} was determined. The speed corresponding to 75% of HR_{max} was calculated.

Tidal breathing flow-volume loops

A fiberglass mask covering both mouth and nostrils was used to support a 15.2-cm diameter pneumotachograph (Merriam Instruments, Grand Rapids, MI). A wire mesh (Mesh SS Screen, McMaster Carr, Chicago, IL) located between the muzzle and pneumotachograph helped to prevent contamination of the pneumotachograph with secretions and served as a flow straightener element. The resistance of the pneumotachograph was 0.04 cm H_2O/L/sec up to an airflow of 90 L/sec. A differential pressure transducer (Model DP45-22, Validyne Sales, Northbridge, CA) measured pressure changes across the pneumotachograph and produced a signal proportional to flow. These signals were then passed through an 8 Hz low-pass filter and fed into the lung function

computer. The computer plotted flow versus volume for each individual breath to create a TBFVL.

Ten representative flow volume loops were selected from each exercise period. Criteria for selection included adequate loop closure (<5% difference between inspiratory and expiratory volume) and lack of artifact such as snorting and swallowing (Amis and Kurpershoek, 1986). A computer software package (Buxco LS-14, Buxco Electronics, Inc., Sharon, CT) was used for quantitative analysis of the flow volume loops. Measured values for individual loops were rate, tidal volume, inspiratory and expiratory time, total inspiratory time, peak inspiratory and expiratory airflow rates, and inspiratory and expiratory flow rates at 50%, 25%, and 12.5% (IF_{50}, IF_{25}, $IF_{12.5}$; EF_{50}, EF_{25}, $EF_{12.5}$) of tidal volume. Ratios of the flow rates and times were also calculated. Heart rate was recorded with a telemetry system (Digital UHF Telemetry System, M1403A, Hewlitt Packard, Palo Alto, CA).

Experimental protocol

Pulmonary resistance and C_{dyn} were measured in standing horses only. Subsequently, horses were exercised on a 3-degree inclined treadmill at 4 m/sec for 2 minutes, followed by a 2-minute exercise period at a treadmill speed corresponding to 75% of the individual HR_{max}. All horses were allowed a 1-minute rest period prior to 2 minutes at treadmill speed corresponding to HR_{max}. Data were collected during the last minute of each 2-minute exercise period. At least fifteen minutes after the last exercise period, each horse was evaluated endoscopically for signs of EIPH. These measurements were taken before, and 5 hours, 1, 3, 5, 7, 14, and 21 days after aerosol

ovalbumin administration. The horses were not exercised at speeds corresponding to HR_{max} five hours after ovalbumin administration because of the severity of clinical signs of respiratory disease.

Statistical analysis

The effect of ovalbumin aerosol challenge on flow-volume loops was analyzed using a repeat measure analysis of variance. Differences between the means were evaluated using Tukey's test (Steele and Torrie, 1960).

Results

Pulmonary function tests

Pulmonary resistance and C_{dyn} in the ovalbumin-sensitized horses before (baseline) and after aerosol ovalbumin administration are shown in Table 1. Five hours after aerosol ovalbumin administration, R_L was significantly increased. It had returned to baseline values by day 1 and remained there for the subsequent measurement periods (Figure 2). Although C_{dyn} was not significantly affected by aerosol ovalbumin administration, the mean C_{dyn} was less than baseline value at the one- and two-day measurement periods. Respiratory frequency was significantly elevated one day after aerosol ovalbumin administration. Tidal volumes were decreased and $\dot{V}_E$ was increased one day after aerosol ovalbumin administration but the changes were not statistically significant (Figure 3).

Table 1. Pulmonary resistance (R_L) and dynamic compliance (C_{dyn}) before (baseline) and after (5 hours, and 1, 3, 5, 7, 14, and 21 days) ovalbumin administration (mean ± SEM).

Measurement period	C_{dyn}	R_L
baseline	1.442 ± 0.19	0.637 ± 0.10
5 hours	1.053 ± 0.20	1.715 ± 0.30*
1 day	0.962 ± 0.35	0.717 ± 0.09
3 days	0.830 ± 0.09	0.745 ± 0.09
5 days	1.976 ± 0.83	0.600 ± 0.09
7 days	1.664 ± 0.23	0.538 ± 0.08
14 days	1.379 ± 0.27	0.703 ± 0.11
21 days	2.112 ± 0.67	0.610 ± 0.11

* = Significant difference ($p < 0.05$).

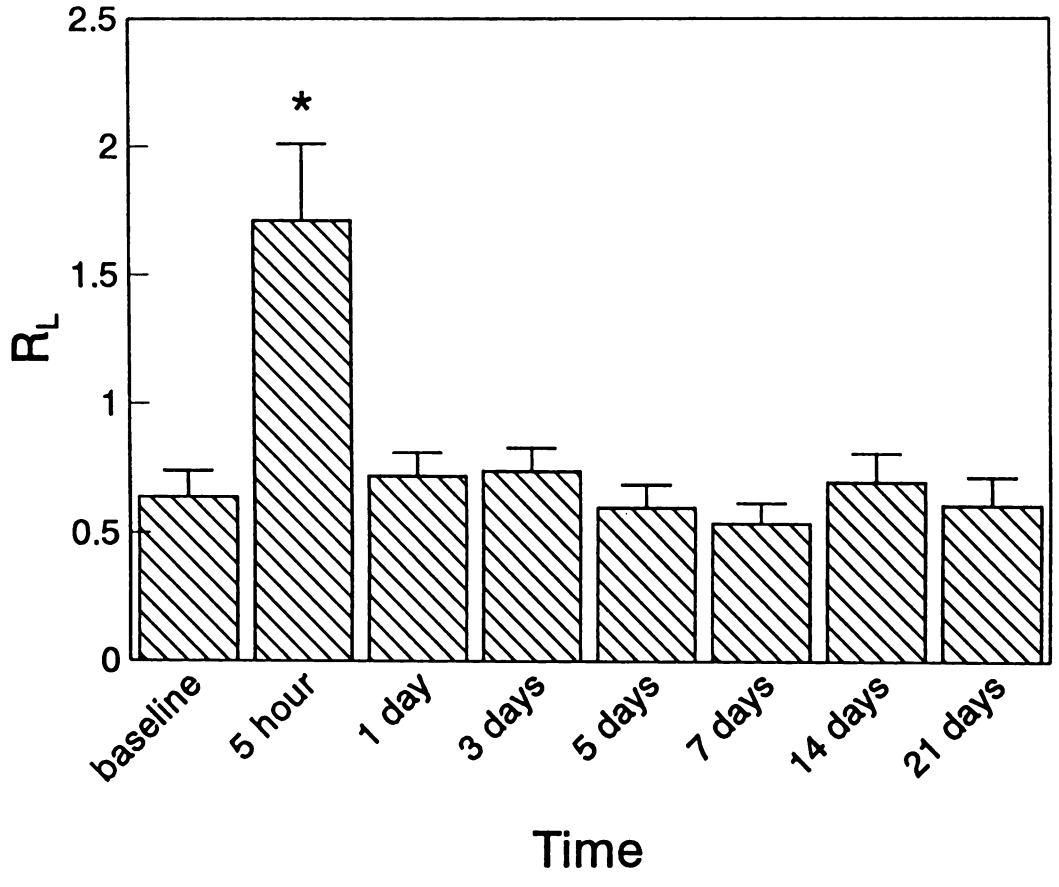

Figure 2. Pulmonary resistance (R_L) (mean ± SEM) measured before (baseline) and after (5 hours, and 1, 3, 5, 7, 14, and 21 days) aerosol ovalbumin administration. * = Significant difference ($p < 0.05$).

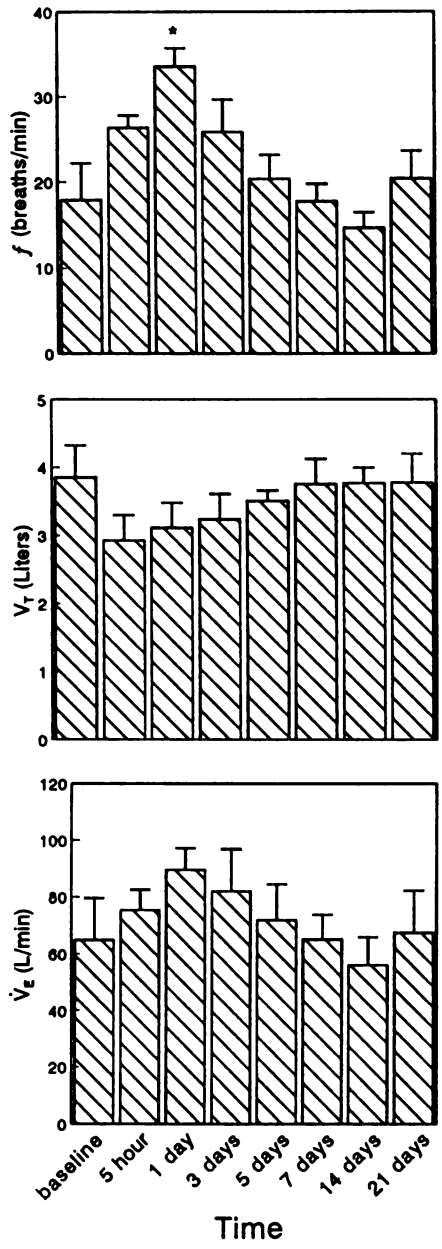

Figure 3. Tidal volume (V_T), respiratory frequency (f), and minute ventilation ($\dot{V}_E$) (mean $\pm$ SEM) measured before (baseline) and after (5 hours, and 1, 3, 5, 7, 14, and 21 days) aerosol ovalbumin administration. * = Significant difference ($p < 0.05$).

Flow-volume loops

The TBFVL indices before (baseline) and after (5 hours, and 1, 3, 5, 7, 14, and 21 days) aerosol ovalbumin administration with horses exercising at speeds corresponding to 75% and 100% of HR_{max} are shown in Tables 2–7. Ovalbumin administration did not result in marked loop changes in any of the horses at any measurement period (Figure 4). Only four indices describing TBFVL were slightly but significantly altered by ovalbumin administration and these indicies are shown in Table 8. At a speed corresponding to 75% HR_{max} the $EF_{12.5}/IF_{12.5}$ was increased at five hours and EF_{25} was increased one day after aerosol ovalbumin administration. When the horses were exercised at speeds corresponding to HR_{max} one day after aerosol ovalbumin administration, IF_{50} and V_T/T_I were significantly decreased from prechallenge values. TBFVL indices were unaffected by aerosol administration of ovalbumin at any of the remaining exercise periods.

Table 2. Rate and timing of the respiratory cycle measured at speeds corresponding to 75% of HR_{max} before (baseline) and after (5 hours, and 1, 3, 5, 7, 14, and 21 days) aerosol ovalbumin administration.

	Measurement periods							
	baseline	5 hours	1 day	3 day	5 day	7 day	14 day	21 day
f (breaths/ min)	97.3 ± 9.78	98.5 ± 9.42	102.7 ± 6.58	101.4 ± 7.74	103.4 ± 8.01	103.4 ± 8.13	100.5 ± 7.25	102.9 ± 7.10
T_I (ms)	324.83 ± 40.68	336.67 ± 43.46	308.50 ± 24.92	317 ± 31.50	294.83 ± 24.75	296.33 ± 26.84	308.33 ± 25.28	297.83 ± 24.09
T_E (ms)	334.50 ± 41.65	322.17 ± 42.83	290.00 ± 19.28	295.50 ± 24.73	306.17 ± 26.62	304.83 ± 26.80	310.00 ± 23.78	303.17 ± 22.80
T_E/T_I	1.03 ± .02	0.96 ± .03	0.95 ± .05	0.95 ± .05	1.04 ± .03	1.04 ± .03	1.02 ± .04	1.03 ± .02
T_I/T_{TOT}	0.49 ± .01	0.51 ± .01	0.51 ± .01	0.52 ± .01	0.49 ± .01	0.49 ± .01	0.50 ± .01	0.49 ± .01
V_T (L)	11.68 ± .94	11.09 ± .70	11.71 ± .84	11.70 ± .78	11.62 ± .81	11.62 ± .83	11.50 ± .84	11.62 ± .83
V_E (L)	11.63 ± .95	10.87 ± .70	11.56 ± .86	11.61 ± .84	11.46 ± .84	11.46 ± .85	11.42 ± .87	11.54 ± .86
V_E/V_I	.99 ± .01	.98 ± .00	.98 ± .01	.99 ± .01	.99 ± .01	.98 ± .01	.99 ± .01	.99 ± .00

f = respiratory frequency, T_I = inspiratory time, T_E = expiratory time, $T_{TOT} = T_I + T_E$, V_T = tidal volume, V_E = expiratory volume, V_I = inspiratory volume.

Table 3. Airflow indices taken from flow-volume loops measured at speeds corresponding to 75% of HR_{max} before (baseline) and after (5 hours, and 1, 3, 5, 7, 14, and 21 days) aerosol ovalbumin administration.

	Measurement periods							
	baseline	5 hours	1 day	3 day	5 day	7 day	14 day	21 day
V_T/T_I	37.27 ± 2.70	34.59 ± 2.53	38.56 ± 2.27	38.01 ± 2.77	39.83 ± 1.42	39.77 ± 2.06	37.91 ± 2.21	39.62 ± 2.10
PIF (L/s)	52.69 ± 3.95	47.60 ± 3.84	59.22 ± 3.20	56.74 ± 3.38	57.84 ± 2.23	57.88 ± 2.44	55.25 ± 2.87	55.97 ± 2.66
IF_{50} (L/s)	43.95 ± 4.78	40.56 ± 3.81	48.01 ± 3.89	47.83 ± 3.97	48.78 ± 2.78	47.20 ± 4.74	44.26 ± 4.04	47.03 ± 3.92
IF_{25} (L/s)	49.96 ± 4.75	45.74 ± 4.32	58.60 ± 3.21	56.15 ± 3.45	56.57 ± 2.61	56.07 ± 3.01	53.09 ± 3.47	54.74 ± 2.84
$IF_{12.5}$ (L/s)	47.19 ± 2.89	41.43 ± 3.41	52.27 ± 2.10	50.33 ± 2.46	51.32 ± 1.57	51.25 ± 1.51	49.29 ± 2.43	49.68 ± 2.31
PEF (L/s)	52.16 ± 1.86	51.36 ± 2.66	58.83 ± 1.20	57.89 ± 1.20	55.49 ± 1.33	54.81 ± 2.00	53.91 ± 1.06	58.24 ± 3.70
EF_{50} (L/s)	44.40 ± 3.25	41.91 ± 2.85	49.74 ± 2.03	50.57 ± 2.11	45.34 ± 2.77	44.94 ± 2.09	45.44 ± 1.25	47.31 ± 2.46
EF_{25} (L/s)	40.37 ± 3.12	41.06 ± 3.05	48.34 ± 1.49	45.71 ± 1.90	42.34 ± 2.89	42.65 ± 3.16	41.60 ± 3.17	40.88 ± 3.40
$EF_{12.5}$ (L/s)	33.17 ± 2.79	35.01 ± 2.46	41.34 ± 1.74	38.81 ± 1.03	35.61 ± 1.39	37.33 ± 1.90	35.44 ± 2.67	35.75 ± 2.64

V_T = tidal volume, T_I = inspiratory time, PIF = peak inspiratory flow, IF_{50} = inspiratory flow at 50% of V_T, IF_{25} = inspiratory flow at 25% of V_T, $IF_{12.5}$ = inspiratory flow at 12.5% of V_T, PEF = peak inspiratory flow, EF_{50} = expiratory flow at 50% of V_T, EF_{25} = expiratory flow at 25% of V_T, $EF_{12.5}$ = expiratory flow at 12.5% of V_T.

Table 4. Ratios of flow indices derived from flow-volume loops measured at speeds corresponding to 75% of HR_{max} before (baseline) and after (5 hours, and 1, 3, 5, 7, 14, and 21 days) aerosol ovalbumin administration.

	Measurement periods							
	baseline	5 hours	1 day	3 day	5 day	7 day	14 day	21 day
PEF/PIF	1.01 ± .05	1.10 ± .04	1.01 ± .06	1.04 ± .06	0.97 ± .03	0.95 ± .04	0.99 ± .04	1.04 ± .05
EF_{50}/IF_{50}	1.06 ± .10	1.07 ± .09	1.08 ± .10	1.10 ± .09	0.94 ± .06	1.00 ± .11	1.07 ± .08	1.05 ± .08
EF_{25}/IF_{25}	0.83 ± .03	0.92 ± .38	0.83 ± .38	0.83 ± .05	0.75 ± .03	0.76 ± .03	0.79 ± .03	0.75 ± .04
$EF_{12.5}/IF_{12.5}$	0.71 ± .03	0.85 ± .02	0.79 ± .04	0.78 ± .04	0.69 ± .02	0.73 ± .02	0.72 ± .03	0.72 ± .03
PIF/IF_{50}	1.23 ± .06	1.19 ± .04	1.27 ± .06	1.21 ± .03	1.20 ± .04	1.28 ± .11	1.29 ± .07	1.23 ± .06
PIF/IF_{25}	1.07 ± .04	1.05 ± .02	1.01 ± .00	1.01 ± .00	1.02 ± .01	1.04 ± .02	1.05 ± .02	1.02 ± .01
$PIF/IF_{12.5}$	1.12 ± .03	1.16 ± .03	1.13 ± .02	1.12 ± .02	1.13 ± .03	1.13 ± .03	1.12 ± .03	1.13 ± .02
IF_{50}/IF_{25}	0.90 ± .06	0.90 ± .05	0.82 ± .03	0.85 ± .02	0.86 ± .03	0.84 ± .07	0.84 ± .05	0.86 ± .03
$IF_{25}/IF_{12.5}$	1.05 ± .05	1.10 ± .03	1.12 ± .03	1.11 ± .02	1.10 ± .04	1.09 ± .04	1.08 ± .04	1.10 ± .03
PEF/EF_{50}	1.21 ± .07	1.24 ± .03	1.19 ± .04	1.15 ± .03	1.25 ± .06	1.23 ± .03	1.19 ± .04	1.23 ± .03
PEF/EF_{25}	1.34 ± .07	1.28 ± .04	1.23 ± .03	1.29 ± .06	1.35 ± .07	1.32 ± .07	1.34 ± .10	1.47 ± .13
$PEF/EF_{12.5}$	1.63 ± .10	1.50 ± .05	1.44 ± .05	1.51 ± .04	1.59 ± .04	1.48 ± .05	1.59 ± .12	1.66 ± .10
EF_{50}/EF_{25}	1.12 ± .05	1.03 ± .04	1.03 ± .03	1.12 ± .05	1.09 ± .03	1.07 ± .03	1.13 ± .08	1.20 ± .11
$EF_{25}/EF_{12.5}$	1.23 ± .05	1.18 ± .02	1.17 ± .02	1.18 ± .03	1.19 ± .04	1.14 ± .03	1.18 ± .03	1.15 ± .04

PEF = peak expiratory flow, PIF = peak inspiratory flow, EF_{50} =expiratory flow at 50% of V_T, EF_{25} = expiratory flow at 25% of V_T, $EF_{12.5}$ = expiratory flow at 12.5% of V_T, IF_{50} = inspiratory flow at 50% of V_T, IF_{25} = inspiratory flow at 25% of V_T, $IF_{12.5}$ = inspiratory flow at 12.5% of V_T.

Table 5. Rate and timing of the respiratory cycle measured at speeds corresponding to 100% of HR_{max} before (baseline) and after (1, 3, 5, 7, 14, and 21 days) aerosol ovalbumin administration.

	Measurement periods						
	baseline	1 day	3 day	5 day	7 day	14 day	21 day
f (breaths/min)	117.4 ± 9.25	108.8 ± 7.47	117.7 ± 10.7	112.8 ± 12.78	119.2 ± 10.93	118.8 ± 12.37	120.2 ± 11.79
T_I (ms)	252.83 ± 28.45	284.73 ± 26.19	259 ± 27.69	274 ± 37.61	250.7 ± 31.25	255.33 ± 39.36	252.5 ± 35.08
T_E (ms)	287.67 ± 22.43	300.73 ± 18.04	281.33 ± 31	304.5 ± 39.84	279.33 ± 26.17	286.83 ± 31.86	278.83 ± 30.71
T_E/T_I	1.17 ± .05	1.09 ± .04	1.10 ± .04	1.13 ± .04	1.14 ± .04	1.15 ± .04	1.12 ± .04
T_I/T_{TOT}	0.46 ± .01	0.48 ± .01	0.48 ± .01	0.47 ± .01	0.47 ± .01	0.47 ± .01	0.47 ± .01
V_T (L)	12.87 ± 1.72	11.86 ± .58	12.27 ± 1.08	13.28 ± 1.7	13.17 ± 1.7	13.04 ± 1.63	12.92 ± 1.60
V_E (L)	12.83 ± 1.78	11.75 ± .62	12.14 ± 1.14	13.18 ± 1.72	13.21 ± 1.72	12.94 ± 1.68	12.88 ± 1.65
V_E/V_I	0.99 ± .01	0.99 ± .01	0.98 ± .01	0.99 ± 00	1.00 ± .01	0.99 ± .01	0.99 ± .01

f = respiratory frequency, T_I = inspiratory time, T_E = expiratory time, $T_{TOT} = T_I + T_E$, V_T = tidal volume, V_E = expiratory volume, V_I = inspiratory volume.

Table 6. Airflow indices taken from flow-volume loops measured at speeds corresponding to 100% of HR_{max} before (baseline) and after (1, 3, 5, 7, 14, and 21 days) aerosol ovalbumin administration.

	Measurement periods						
	baseline	1 day	3 day	5 day	7 day	14 day	21 day
V_T/T_I	51.16 ± 2.46	44.04 ± 2.11	48.61 ± 2.32	49.16 ± 1.83	52.58 ± 1.86	52.15 ± 2.62	51.81 ± 2.62
PIF (L/s)	74.77 ± 3.86	67.94 ± 2.19	70.72 ± 3.73	72.80 ± 2.67	75.60 ± 3.25	74.63 ± 3.17	75.19 ± 3.08
IF_{50} (L/s)	67.25 ± 3.33	58.21 ± 3.9	62.31 ± 4.19	64.92 ± 2.54	65.41 ± 4.47	67.05 ± 3.75	66.52 ± 2.38
IF_{25} (L/s)	73.41 ± 3.63	66.45 ± 2.45	69.09 ± 3.77	71.33 ± 2.56	73.79 ± 3.06	72.88 ± 3.07	72.64 ± 4.32
$IF_{12.5}$ (L/s)	64.13 ± 4.04	58.70 ± 1.16	61.16 ± 3.17	63.11 ± 3.09	65.57 ± 3.36	64.37 ± 2.65	62.28 ± 5.88
PEF (L/s)	65.43 ± 5.3	58.01 ± 2.01	65.34 ± 3.66	64.61 ± 3.59	69.05 ± 4.03	65.91 ± 3.27	66.23 ± 2.80
EF_{50} (L/s)	57.50 ± 4.98	53.12 ± 1.46	57.75 ± 4.07	56.27 ± 3.52	60.35 ± 4.25	55.91 ± 2.83	58.50 ± 1.84
EF_{25} (L/s)	48.47 ± 3.68	46.78 ± 1.43	49.09 ± 3.27	47.72 ± 3.69	51.79 ± 4.24	48.97 ± 2.85	48.79 ± 3.91
$EF_{12.5}$ (L/s)	43.56 ± 3.27	41.19 ± 1.13	42.01 ± 3.24	42.59 ± 2.55	47.59 ± 3.75	44.03 ± 2.41	44.75 ± 1.88

V_T = tidal volume, T_I = inspiratory time, PIF = peak inspiratory flow, IF_{50} = inspiratory flow at 50% of V_T, IF_{25} = inspiratory flow at 25% of V_T, $IF_{12.5}$ = inspiratory flow at 12.5% of V_T, PEF = peak inspiratory flow, EF_{50} = expiratory flow at 50% of V_T, EF_{25} = expiratory flow at 25% of V_T, $EF_{12.5}$ = expiratory flow at 12.5% of V_T.

Table 7. Ratios of flow indices derived from flow-volume loops measured at speeds corresponding to 100% of HR_{max} before (baseline) and after (1, 3, 5, 7, 14, and 21 days) aerosol ovalbumin administration.

	Measurement periods						
	baseline	1 day	3 day	5 day	7 day	14 day	21 day
PEF/PIF	0.88 ± .05	0.87 ± .06	0.93 ± .05	0.89 ± .04	0.92 ± .04	0.89 ± .04	0.88 ± .02
EF_{50}/IF_{50}	0.85 ± .05	0.99 ± .10	0.94 ± .06	0.87 ± .05	0.93 ± .06	0.84 ± .03	0.88 ± .03
EF_{25}/IF_{25}	0.66 ± .02	0.72 ± .02	0.73 ± .06	0.67 ± .04	0.70 ± .04	0.67 ± .03	0.68 ± .04
$EF_{12.5}/IF_{12.5}$	0.68 ± .01	0.70 ± .02	0.70 ± .05	0.68 ± .02	0.72 ± .03	0.68 ± .02	0.75 ± .07
PIF/IF_{50}	1.12 ± .02	1.21 ± .05	1.15 ± .03	1.12 ± .02	1.18 ± .06	1.12 ± .02	1.13 ± .04
PIF/IF_{25}	1.02 ± 0.0	1.03 ± .01	1.03 ± .01	1.02 ± .01	1.03 ± .01	1.03 ± 0.0	1.04 ± .03
$PIF/IF_{12.5}$	1.17 ± .03	1.16 ± .02	1.16 ± .03	1.16 ± .03	1.16 ± .03	1.16 ± .03	1.26 ± .11
IF_{50}/IF_{25}	0.92 ± .02	0.87 ± .02	0.90 ± .02	0.91 ± .01	0.89 ± .04	0.92 ± .02	0.93 ± .05
$IF_{25}/IF_{12.5}$	1.15 ± .03	1.13 ± .03	1.13 ± .03	1.14 ± .04	1.13 ± .03	1.14 ± .03	1.20 ± .07
PEF/EF_{50}	1.15 ± .04	1.10 ± .02	1.15 ± .04	1.16 ± .03	1.16 ± .05	1.19 ± .06	1.14 ± .04
PEF/EF_{25}	1.36 ± .07	1.25 ± .06	1.35 ± .07	1.39 ± .06	1.37 ± .08	1.37 ± .09	1.40 ± .13
$PEF/EF_{12.5}$	1.52 ± .06	1.42 ± .06	1.60 ± .10	1.54 ± .03	1.49 ± .08	1.53 ± .10	1.51 ± .07
EF_{50}/EF_{25}	1.19 ± .04	1.14 ± .05	1.19 ± .05	1.20 ± .03	1.19 ± .04	1.16 ± .05	1.24 ± .09
$EF_{25}/EF_{12.5}$	1.12 ± .02	1.14 ± .01	1.18 ± .02	1.12 ± .04	1.10 ± .06	1.11 ± .02	1.10 ± .08

PEF = peak expiratory flow, PIF = peak inspiratory flow, EF_{50} =expiratory flow at 50% of V_T, EF_{25} = expiratory flow at 25% of V_T, $EF_{12.5}$ = expiratory flow at 12.5% of V_T, IF_{50} = inspiratory flow at 50% of V_T, IF_{25} = inspiratory flow at 25% of V_T, $IF_{12.5}$ = inspiratory flow at 12.5% of V_T.

Table 8. Flow-volume loop variables measured at speeds corresponding to 75% (75% HR_{max}) and 100% (100% HR_{max}) of maximum heart rate, which changed significantly after ovalbumin administration (mean ± SEM).

	Measurement periods							
	baseline	5 hours	1 day	3 day	5 day	7 day	14 day	21 day
75% HR_{max}								
$EF_{12.5}/IF_{12.5}$	0.707 ± 0.03	0.850* ± 0.02	0.795 ± 0.04	0.783 ± 0.04	0.695 ± 0.02	0.727 ± 0.02	0.717 ± 0.03	0.718 ± 0.03
EF_{25}	40.37 ± 3.12	41.06 ± 3.05	48.34* ± 1.49	45.71 ± 1.90	42.34 ± 2.89	42.65 ± 3.16	41.60 ± 3.17	40.88 ± 3.40
100% HR_{max}								
V_T/T_I	51.16 ± 2.46	—	44.04* ± 2.11	48.61 ± 2.32	49.16 ± 1.83	52.58 ± 1.86	52.15 ± 2.62	51.81 ± 2.62
IF_{50}	67.25 ± 3.33	—	58.21* ± 3.90	62.31 ± 4.19	64.92 ± 2.54	65.41 ± 4.47	67.05 ± 3.75	66.52 ± 2.38

* = Significance at $p < 0.05$; $EF_{12.5}$ = expiratory flow at 12.5% of V_T, $IF_{12.5}$ = inspiratory flow at 12.5% of V_T, EF_{25} = expiratory flow at 25% of V_T, V_T = tidal volume, T_I = inspiratory time, IF_{50} = inspiratory flow at 50% of V_T.

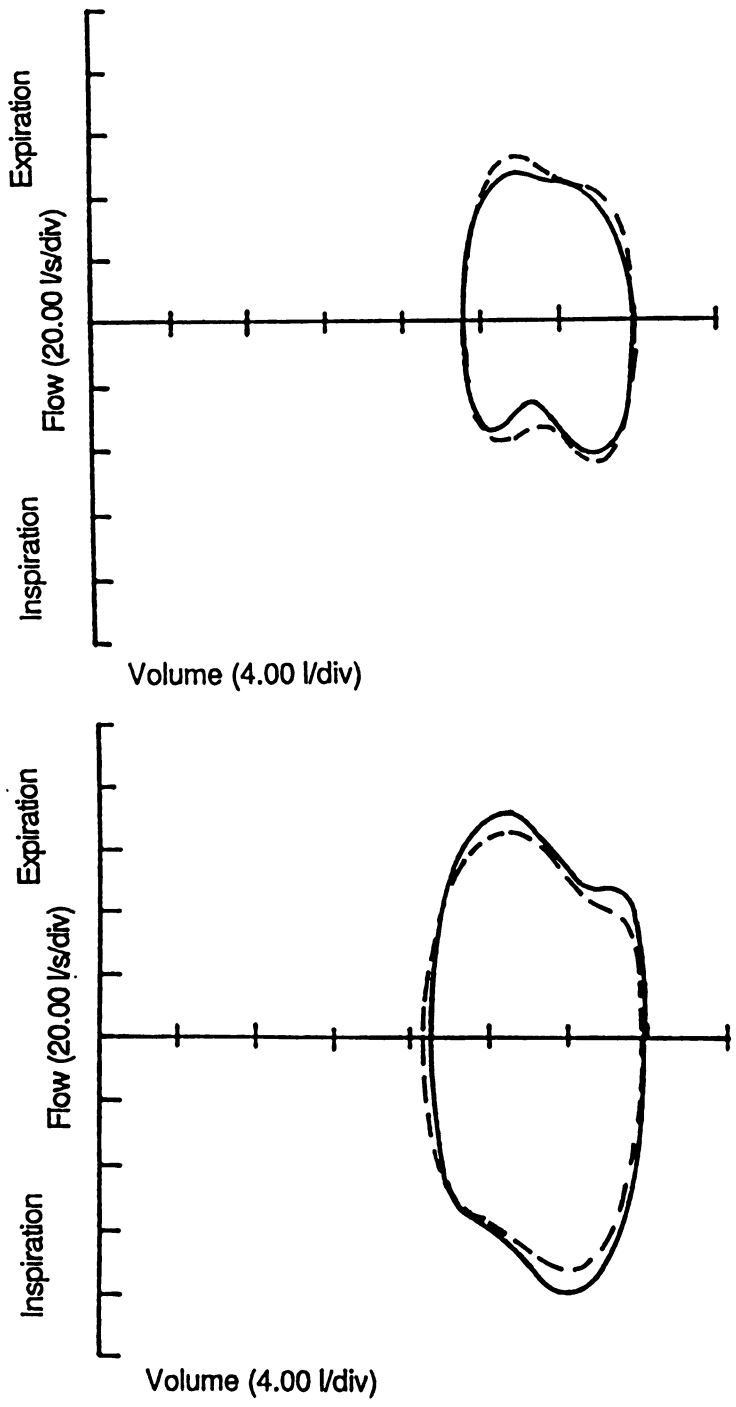

Figure 4. Representative flow-volume loops measured before (solid line) and after (broken line) aerosol ovalbumin administration. The upper loops are measured at 75% of HR_{max}. The broken line is five hours after ovalbumin administration. The lower loops are measured at HR_{max}. The broken line is one day after ovalbumin administration.

Heart rate

Heart rate was significantly elevated at five hours after aerosol ovalbumin administration when the horses were running at speeds corresponding to 75% of HR_{max} (Figure 5). This elevated rate was equal to the mean baseline HR_{max} for the horses in this study.

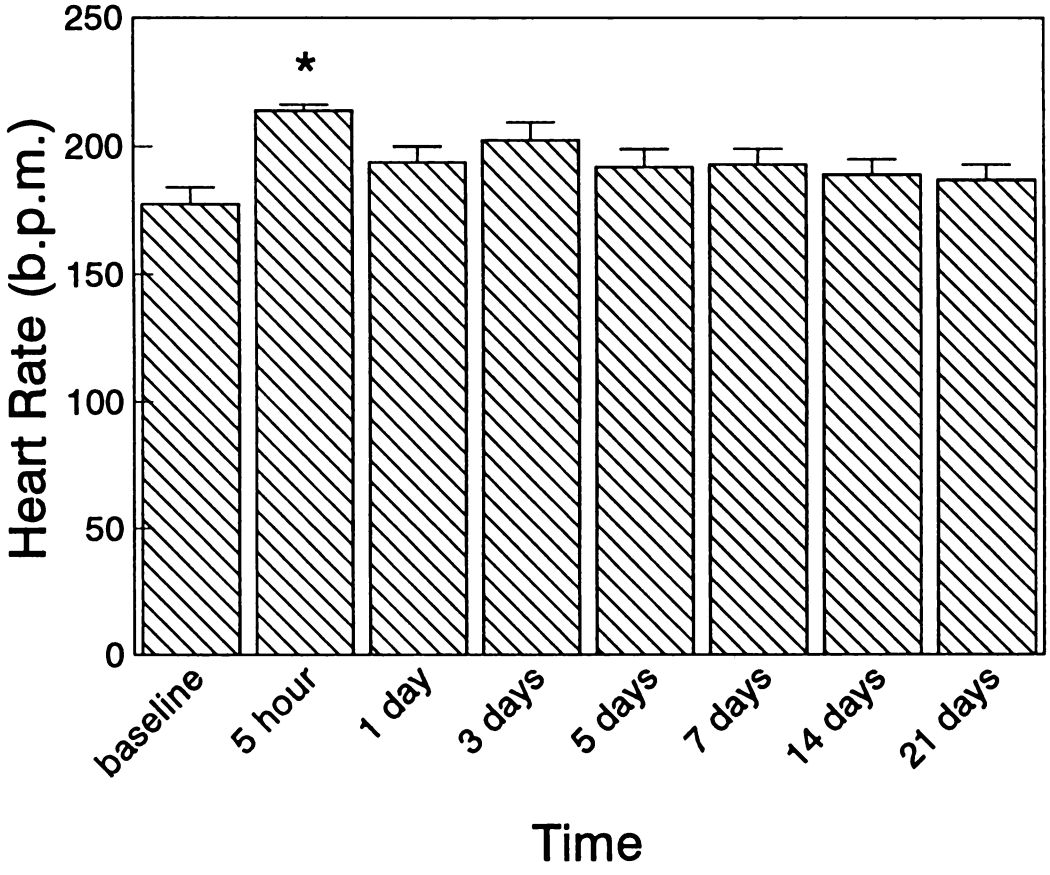

Figure 5. Heart rate (mean ± SEM) measured at treadmill speeds corresponding to 75% HR_{max} before (baseline) and after (5 hours and 1, 3, 5, 7, 14, and 21 days) aerosol ovalbumin administration. * = significant difference ($p < 0.05$).

Endoscopic examination

Endoscopic evaluation at least fifteen minutes after the last exercise period revealed the presence of EIPH in all of the horses at 5 hours and at one day after ovalbumin administration. In four of the six horses EIPH was also observed on day three. Exercise-induced pulmonary hemorrhage was not observed after exercise at any of the remaining measurement periods.

Discussion

Administration of ovalbumin aerosol to sensitized horses resulted in airway obstruction, indicated by an approximately two-fold increase in R_L and a smaller decrease in C_{dyn}. Similar pulmonary function changes in response to ovalbumin aerosol challenge to sensitized ponies has been previously reported. In sensitized horses, aerosol ovalbumin challenge causes acute fibrinopurulent obstructive bronchiolitis, and bronchitis with pulmonary congestion and hemorrhage. Thus the airway narrowing observed is due primarily to airway obstruction with exudate, edema fluid, and hemorrhage (Derksen et al., 1982b).

In human medicine, MEFVL analysis is a commonly used, noninvasive, and sensitive test used to detect airway obstruction (Hyatt and Black, 1973). The loop is a ventilatory function test, whereby a continuous graphic measure of air flow vs. volume is plotted for a single maximum inspiratory and expiratory effort. The test requires patient cooperation to perform this maneuver (Bass, 1973). In veterinary medicine the noncooperative nature of our patients has prevented the clinical use of the MEFVL. In noncooperative subjects such as dogs (Amis and Kurpershoek, 1986), cats (McKiernan

et al.), and human neonates (Godfrey et al., 1983), TBFVLs have been used to evaluate airway obstruction. Tidal breathing flow-volume loops have lower than maximum flow rates and greater flow variability because of the voluntary control over breathing patterns. These factors contribute to the lack of sensitivity of TBFVLs in the detection of airway obstruction.

Horses exercising maximally generate near-maximum flow rates. Lumbsden et al. recently reported that TBFVL analysis in exercising horses is a quantitative, specific, and repeatable test in the detection of upper airway obstruction. We reasoned that tidal breathing flow-volume loop analysis in exercising horses may also be used to detect the presence of intrathoracic airway obstruction. We expected that TBFVL analysis generated in exercising horses after ovalbumin challenge would be significantly different from TBFVL in the same horses before ovalbumin challenge and that this difference would be detectable for several days after the R_L had returned to baseline values. Figure 4 illustrates that in horses with airway obstruction, exercising at 75% or 100% of HR_{max}, the shape of the TBFVL was not markedly different from normal horses exercising at the same speeds. Both inspiratory and expiratory flow patterns were not markedly affected by airway obstruction. Similar conclusions are reached by evaluation of quantitative indices describing loop shape and size. Although the indices listed in Table 3 were significantly altered by airway obstruction, the changes were small numerically.

What could have caused our failure to detect airway obstruction in exercising horses with airway obstruction? The increase in R_L and decrease in C_{dyn} in standing horses after aerosol ovalbumin administration suggests the presence of detectable airway obstruction. It is possible that this airway obstruction was alleviated by exercise itself.

This possibility is unlikely because the mechanism of ovalbumin-induced airway obstruction in sensitized horses involved physical factors such as exudate, edema, and hemorrhage, rather than reflex mechanisms or smooth muscle contraction (Derksen et al., 1982b). It is unlikely that exercise resolved this inflammatory process. Indeed, following exercise in all horses EIPH was observed at the 5-hour and 1-day measurement periods. If EIPH occurred during exercise the blood may have exacerbated already existing airway obstruction.

In humans, supreme athletes employ near-maximum air flows during maximal exercise (Grimby et al., 1971). In contrast, in sedentary persons airflow during maximum exercise is significantly less than maximum achievable air flows. It is possible that normal horses also use less than maximally achievable air flows during high-intensity exercise. If this is so, significant reduction in maximal air flows may not be detected unless the obstruction is severe.

In the present study we did not measure EELV. In exercising humans EELV stays constant or is reduced (Henke et al., 1988). The advantage of this breathing strategy is that it places the diaphragm in a more optimal part of its length-tension curve, improving the mechanical advantage of the inspiratory muscles and reducing the elastic work of inspiratory muscles. When this breathing strategy is employed, subjects impinge on their maximal expiratory flow-volume curve near end expiration. In contrast, EELV at maximal exercise is increased in patients with mild-to-moderate airway obstruction (Babb et al., 1991). In these patients, maximal expiratory flow at low lung volumes is very low. Therefore, increasing EELV to a level where maximal expiratory flow is higher increases the mean expiratory flow rates, and thus ventilatory capacity. The cost

of this breathing strategy is an increased work of breathing. Five hours following the induction of airway obstruction, HR at the first exercise period was significantly increased, and indeed was maximum. This suggests that the work of breathing in these horses may have been increased. Increase in work of breathing may have been due to airway obstruction itself, but also may be due to an increase in EELV allowing high air flow rates in spite of airway obstruction.

Aerosol ovalbumin administration to sensitized horses resulted in a significant increase in *f*. Similar changes have been reported previously in ponies. Because in ponies the tachypnea is alleviated by vagal blockade, this ventilatory response to inhaled antigen is due to stimulation of pulmonary receptors with vagal afferent fibers (Derksen et al., 1982b).

In conclusion, aerosol ovalbumin administration to sensitized horses caused moderate airway obstruction. Evaluation of TBFVLs generated in these horses exercising at 75 and 100% of their HR_{max} failed to detect this airway obstruction.

SUMMARY AND CONCLUSIONS

The purpose of this study was to evaluate the usefulness of TBFVLs in evaluating pulmonary function in the exercising horse. Maximum expiratory flow-volume loops have been used as a noninvasive and sensitive test for the evaluation of airway obstruction in humans. Because the horse is a noncooperative patient, our measurements were taken during intense exercise to increase airflow. This not only served to more closely approximate airflows achieved with MEFVLs, but also simulated the conditions under which exercise-limiting respiratory dysfunction becomes clinically detectable.

Administration of aerosol ovalbumin to previously sensitized horses resulted in airway obstruction, which was measured by increased R_L and decreased C_{dyn}. This airway obstruction was not detected by TBFVL analysis.

We did not measure EELV in these horses during exercise. In healthy, exercising humans EELV is decreased or remains constant. However in patients with mild-to-moderate airflow limitation, EELV increases. By increasing lung volume, the maximum expiratory flow would be higher. This allows higher expiratory flow rates and thus an increase in ventilatory capacity. It is possible that this is why we were unable to detect airway obstruction with TBFVL analysis.

References

REFERENCES

Amis TC, Kurpershoek C. (1986) Tidal breathing flow-volume loop analysis for clinical assessment of airway obstruction in conscious dogs. *Am J Vet Res* 47(5):1002-1006.

Art T, Lekeux P. (1988) Respiratory airflow patterns in ponies at rest and during exercise. *Can J Vet Res* 52:299-303.

Babb TG, Viggiano R, Hurley B, Staats B, Rodarte JR. (1991) Effect of mild-to-moderate airflow limitation on exercise capacity. *J Appl Physiol* 70(1):223-230.

Bass J. (1973) The flow-volume loop: normal standards and abnormalities in chronic obstructive pulmonary disease. *Chest* 63(2):171-176.

Bayly WM, Slocombe RF. (1991) Ventilatory responses of horses to exercise. *Eq Vet Sci* 11(5):288-293.

Belknap JK, Derksen FJ, Nickels FA, Stick JA, Robinson NE. (1990) Failure of subtotal arytenoidectomy to improve upper airway flow mechanics in exercising Standardbreds with induced aryngeal hemiplegia. *Am J Vet Res* 51(9):1481-1487.

Bianco S, Vaghi A, Robuschi M, Pasargihlian M. (1988) Prevention of exercise-induced bronchoconstriction by inhaled frusemide. *Lancet* 1988:252-255.

Broadstone RV, Robinson NE, Berney C, Benson C, Derksen FJ. (1990) Furosemide attenuates airway obstruction in ponies. *FASEB* Abstract (April).

Castile R, Mead J, Jackson A, Wohl ME, Stokes D. (1979) Effects of posture on flow-volume curve configuration in normal humans. *J Appl Physiol* 46:565-570.

Chan-Yeung M. (1973) Maximal expiratory flow and airway resistance during induced bronchoconstriction in patients with asthma due to western red cedar (*thuja plicata*). *Am Rev Respir Dis* 108:1103-1109.

Davis JL, Manohar M. (1988) Effect of splenectomy on exercise-induced pulmonary and systemic hypertension in ponies. *Am J Vet Res* 49(7):1169-1172.

Dawson SV, Elliott EA. (1977) Wave-speed limitation on expiratory flow—a unifying concept. *J Appl Physiol* 43(3):498-515.

Derksen FJ, Robinson NE, Slocombe RF. (1982) Ovalbumin-induced lung disease in the pony: role of vagal mechanisms. *J Appl Physiol* 53:719-725.

Derksen FJ, Robinson NE, Slocombe RF, et al. (1982) Pulmonary function in standing ponies: reproducibility and effect of vagal blockade. *Am J Vet Res* 43:598-602.

Derksen FJ, Slocombe RF, Gray PR, Robinson NE. (1992) Exercise-induced pulmonary hemorrhage in horses with experimental allergic lung disease. *Am J Vet Res* 53(1):15-21.

Erickson BK, Erickson HH, Coffman JR. (1989) Exercise-induced pulmonary hemorrhage during high intensity exercise: potential causes and the role of furosemide. *American Association of Equine Practitioners* 35:375-379.

Erickson BK, Erickson HH, Coffman JR. (1992) Pulmonary artery and aortic pressure changes during high intensity treadmill exercise in the horse: effect of frusemide and phentolamine. *Eq Vet J* 24(3):215-219.

Evans DL, Rose RJ. (1983) Maximum oxygen uptake in racehorses: changes with training state and prediction from submaximal cardiorespiratory measurements. In: *Equine Exercise Physiology 2*, eds. Gillespie JR, Robinson NE. ICEEP Publications, Davis, CA, pp. 52-65.

Evans DL, Rose RJ. (1988) Cardiovascular and respiratory responses in Thoroughbred horses during treadmill exercise. *J Exp Biol* 134:397-408.

Freestone JF, Carlson GP, Harrold DR, Church G. (1988) Influence of furosemide treatment on fluid and electrolyte balance in horses. *Am J Vet Res* 49(11):1899-190.

Fry DL, Hyatt RE. (1960) Pulmonary mechanics: a unifying analysis of the relationsip between pressure, volume and gasflow in the lungs of normal and diseased human subjects. *Am J Med* 29:672-689.

Geor RJ, Weiss DJ, Burris SM, Smith CM. (1992) Effects of furosemide and pentoxifylline on blood flow properties in horses. *Am J Vet Res* 53(11):2043-2049.

Godfrey S, Bar-Yishay E, Arad I, Landau LI, Taussig LM. (1983) Flow-volume curves in infants with lung disease. *Pediatrics* 72(4):517-522.

Goetz TE, Manohar M. (1986) Pressures in the right side of the heart and esophagus (pleura) in ponies during exercise before and after furosemide administration. *Am J Vet Res* 47(2):270-276.

Green M, Mead J, Turner JM. (1974) Variability of maximum expiratory flow-volume curves. *J Appl Physiol* 37(1):67-74.

Grimby G, Saltin B, Wilhelmsen L. (1971) Pulmonary flow-volume and pressure-volume relationship during submaximal and maximal exercise in young well-trained men. *Bull Physiol Pathol Respir* 7:157-168.

Henke KG, Sharratt M, Pegelow D, Dempsey JA. (1988) Regulation of end-expiratory lung volume during exercise. *J Appl Physiol* 64(1):135-146

Hillidge CJ, Lane TJ, Whitlock BS. (1985) Exercise-induced pulmonary hemorrhage in the racing appaloosa horse. *J Eq Vet Sci* 5(6):351-353.

Hillidge CJ, Lane TJ, Johnson EL, Asquith RL. (1984) Preliminary investigations of exercise-induced pulmonary hemorrhage in racing quarter horses. *J Eq Vet Sci* 4(1):21-23.

Hyatt RE, Schilder DP, Fry DL. (1958) Relationship between maximum expiratory flow and degree of lung inflation. *J Appl Physiol* 13:331-336.

Hyatt RE, Black LF. (1973) The flow-volume curve. a current perspective. *Am Rev Respir Dis* 107:191-197.

Hyatt RE, Rodarte JR, Mead J, Wilson. (1979) Changes in lung mechanics: flow-volume relations. In: *Lung Biology in Health and Disease: The Lung in the Transition*

Between Health and Disease, eds. PT Macklem and S Permutt. New York: Dekker, vol. 12, pp. 73-112.

Hyatt RE. (1986) Forced expiration. In: *Handbook of Physiology. The Respiratory System*, eds. AP Fishman, PT Macklem, J Mead, SR Geiger, Bethesda, MD: Am. Physiol. Soc. Sec. 3, vol. III, chap 19, p. 295-314.

Ingram RH, Schilder DP. (1966) Effect of gas compression on pulmonary pressure, flow, and volume relationship. *J Appl Physiol* 21(6):1821-1826.

Jordanoglou J, Pride NB. (1968) A comparison of maximum inspiratory and expiratory flow in health and in lung disease. *Thorax* 23:38-45.

Knudson RJ, Slatin RC, Lebowitz MD, Burrows B. (1976) The maximum expiratory flow-volume curve: normal standards, variability, and effects of age. *Am Rev Respir Dis* 113:587-600.

Lumsden JM, Derksen FJ, Stick JA, Robinson NE. Evaluation of upper airway obstruction in the exercising horse using flow-volume loops. In preparation for publication.

MacNamara B, Bauer S, Iafe J. (1990) Endoscopic evaluation of exercise-induced pulmonary hemorrhage and chronic obstructive pulmonary disease in association with poor performance in racing Standardbreds. *JAVMA* 196(3):443-445.

Mahaffey LW. (1962) Respiratory conditions in horses. *Vet Rec* 74:1295-1314.

Manohar M. Exercise-induced pulmonary hemorrhage in horses: pulmonary vascular pressures, furosemide, and bronchial circulation. Seminar presented at Michigan State University, October 27, 1992.

McClay CB, Weiss DJ, Smith CM, Gordon B. (1992) Evaluation of hemorheologic variables as implications for exercise-induced pulmonary hemorrhage in racing thoroughbreds. *Am J Vet Res* 53(8):1380-1385.

McKiernan BC, Dye JA, Rozanski, EA. Tidal breathing flow-volume loops (TBFVL) in healthy and bronchitic cats. In preparation for submission.

Mead J, Turner JM, Macklem PT, Little JB. (1967) Significance of the relationship between lung recoil and maximum expiratory flow. *J Appl Physiol* 22(1):95-108.

Mead J. (1978) Analysis of the configuration of maximum expiratory flow-volume curves. *J Appl Physiol* 44(2):156-165.

Milic-Emili J, Henderson JAM, Dolovich MB, Trop D, Kaneko K. (1966) Regional distribution of inspired gas in the lung. *J Appl Physiol* 21:749.

Morris EA, Seeherman HJ. (1991) Clinical evaluation of poor performance in the racehorse: the results of 275 evaluations. *Equine Vet J* 23:169-174.

O'Callaghan MW, Pascoe JR, Tyler WS, Mason DK. (1987a) Exercise-induced pulmonary hemorrhage in the horse: results of a detailed clinical, post mortem and imaging study. I. Clinical profile of horses. *Eq Vet J* 19(5):384-388.

O'Callaghan MW, Pascoe JR, O'brien TR, Hornof WJ, Mason DK. (1987b) Exercise-induced pulmonary hemorrhage in the horse: results of a detailed clinical, post mortem and imaging study. VI. Radiological/pathological correlations. *Eq Vet J* 19(5):419-422.

O'Callaghan MW, Pascoe JR, Tyler WS, Mason DK. (1987c) Exercise-induced pulmonary hemorrhage in the horse: results of a detailed clinical, post mortem and imaging study. II. Gross lung pathology. *Eq Vet J* 19(5):389-393.

O'Callaghan MW, Pascoe JR, Tyler WS, Mason DK. (1987d) Exercise-induced pulmonary hemorrhage in the horse: results of a detailed clinical, post mortem and imaging study. III. Subgross findings in lungs subjected to latex perfusions of the bronchial and pulmonary arteries. *Eq Vet J* 19(5):394-404.

O'Callaghan MW, Pascoe JR, Tyler WS, Mason DK. (1987e) Exercise-induced pulmonary hemorrhage in the horse: results of a detailed clinical, post mortem and imaging study. V. Microscopic observations. *Eq Vet J* 19(5):411-418.

O'Callaghan MW, Pascoe JR, Tyler WS, Mason DK. (1987f) Exercise-induced pulmonary hemorrhage in the horse: results of a detailed clinical, post mortem and imaging study. IV. Changes in the bronchial circulation demonstrated by C. T. scanning and microradiography. *Eq Vet J* 19(5):405-410.

O'Callaghan MW, Hornof WJ, Fisher PE, Pascoe JR. (1987g) Exercise-induced pulmonary hemorrhage in the horse: results of a detailed clinical, post mortem and imaging study. VII. Ventilation/perfusion scintigraphy in horses with EIPH. *Eq Vet J* 19(5):423-427.

O'Callaghan MW, Pascoe JR, Tyler WS, Mason DK. (1987h) Exercise-induced pulmonary hemorrhage in the horse: results of a detailed clinical, post mortem and imaging study. VIII. Conclusions and implications. *Eq Vet J* 19(5):428-434.

Olafsson S, Hyatt RE. (1969) Ventilatory mechanics and expiratory flow limitation during exercise in normal subjects. *J Clin Invest* 48:564-573.

Olsen SC, Coyne CP, Lowe BS, Pelletier N, Raub EM, Erickson HH. (1992) Influence of cyclooxygenase inhibitors on furosemide-induced hemodynamic effects during exercise in horses. *Am J Vet Res* 53(9):1562-1567.

Pascoe JR, Ferraro GL, Cannon JH, Arthur RM, Wheat JD. (1981) Exercise-induced pulmonary hemorrhage in racing Thoroughbreds: a preliminary study. *Am J Vet Res* 42:703-707.

Pascoe JR, McCabe AE, Franti CE, Arthur RM. (1985) Efficacy of furosemide in the treatment of exercise-induced pulmonary hemorrhage in Thoroughbred racehorses. *Am J Vet Res* 46(9):2000-2003.

Pascoe JR, O'Brien TR, Wheat JD, Meagher DM. (1987) Radiographic aspects of exercise-induced pulmonary hemorrhage in racing horses. *Vet Rad* 24(2):85-92.

Pascoe JR. (1989) Exercise-induced pulmonary hemorrhage. *J Eq Vet Sci* 9(4):198-200.

Peslin R, Bohadana A, Hannhart B, Jardin P. (1979) Comparison of various methods for reading maximal expiratory flow-volume curves. *Am Rev Respir Dis* 119:271-277.

Petsche VM, Derksen FJ, Robinson NE. Flow-volume loops in horses with recurrent airway obstruction (heaves). In preparation for submission.

Pollmann U, Hörnicke H. (1987) Characteristics of respiratory airflow during exercise in horses with reduced performance due to pulmonary emphysema or bronchitis. In: *Equine Exercise Physiology 2*, eds. Gillespie JR, Robinson NE, Davis, CA: ICEEP Publications, pp. 760-771.

Potter WA, Olafsson S, Hyatt RE. (1971) Ventilatory mechanics and expiratory flow limitation during exercise in patients with obstructive lung disease. *J Clin Invest* 50:910-919.

Pride NB, Permutt S, Riley RL, Bromberger-Barnea B. (1967) Determinants of maximal expiratory flow from the lungs. *J Appl Physiol* 23(5): 646-662.

Raphel CF, Soma LR. (1982) Exercise-induced pulmonary hemorrhage in thoroughbreds after racing and breezing. *Am J Vet Res* 43(7):1123-1127.

Robinson NE, Derksen FJ. (1981) Small airway obstruction as a cause of exercise-associated pulmonary hemorrhage: an hypothesis. *American Association of Equine Practitioners* 26:421-430.

Rose RJ, Hendrickson DK, Knight PK. (1990) Clinical exercise testing in the normal Thoroughbred racehorse. *Australian Vet J* 67:345-348.

Steele RGD, Torrie JH. (1960) *Principles and Procedures of Statistics.* New York: McGraw-Hill Book Co.

Stubbing DG, Pengelly LD, Morse JLC, Jones NL. (1980a) Pulmonary mechanics during exercise in normal males. *J Appl Physiol* 49(3):506-510.

Stubbing DG, Pengelley LD, Morse JLC, Jones NL. (1980b) Pulmonary mechanics during exercise in subjects with chronic airflow obstruction. *J Appl Physiol* 49(3):511-515.

Sweeney CR, Soma LR, Maxson AD, Thompson JE, Holcombe SJ, Spencer Pa. (1990) Effects of furosemide on the racing times of Thoroughbreds. *Am J Vet Res* 51(5):772-778.

Sweeney CR. (1991) Exercise-induced pulmonary hemorrhage. *Vet Clin North Am Eq Pract* 7(1):93-104.

Wasserman K, Hansem JE, Sue DY, Whipp BJ. (1987) *Principles of Exercise Testing and Interpretation.* Philadelphia: Lea and Febiger.

Weber KT, Janicki JS, McElroy PA, Reddy HK. (1988) Concepts and applications of cardiopulmonary exercise testing. *Chest* 93:843-847.

West JB, Tsukimoto K, Mathieu-Costello O, Prediletto R. (1991) Stress failure in pulmonary capillaries. *J Appl Physiol* 70(4):1731-1742.

West JB, Mathieu-Costello O, Logemann RB, Jones JH, Pascoe JR, Tyler WS. (1992) Stress failure of pulmonary capillaries in racehorses with exercise-induced pulmonary hemorrhage. *FASEB J* 6(5);A2048.

Wise PH, Krauss AN, Waldman S, Auld PA. (1980) Flow-volume loops in newborn infants. *Critical Care Med* 8(2):61-63.

Younes M, Kivinen G. (1984) Respiratory mechanics and breathing pattern during and following maximal exercise. *J Appl Physiol* 57(6):1773-1782.