# EVALUATION OF RESPIRATORY INDUCTANCE PLETHYSMOGRAPHY IN HEAVES-AFFECTED HORSES

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## ABSTRACT

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Recurrent airway obstruction (RAO) is an airway disease that becomes clinically evident in susceptible middle-aged horses. The obstruction by bronchospasm and excess mucoid secretions is a consequence of inflammation that occurs within a few hours of inhalation of organic dust. The onset and progression of the disease is difficult to monitor with the standard methods used to assess pulmonary function in horses. Therefore, the main objective is to use non-invasive respiratory inductance plethysmography (RIP) to investigate differences in breathing pattern between control and RAO-affected horses during the onset of airway obstruction induced by stabling. Pulmonary function indicated airway obstruction in the RAO groups on the final two days of stabling. The values for RIP (RIPf and RIPtd) did not differ between groups, but the standard deviation of these decreased significantly within eight hours of the onset of stabling in RAO-affected horses. RIP output and pulmonary function were highly correlated as severity of disease progressed. The decrease in standard deviation of RIP output indicates decreased breathing pattern variability in RAO- affected horses. This loss of variability may provide an early indicator of airway inflammation.

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## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

# INTRODUCTION

This thesis contains three chapters. The first chapter is a literature review that describes an obstructive pulmonary disease in horses termed recurrent airway obstruction. A brief breakdown of previous terminology used to describe this disease will aid in the understanding of the current knowledge of the pathophysiology responsible for the loss in pulmonary function in affected horses. The loss of pulmonary function in horses is evaluated by a standard method used to measure lung mechanics variables such as changes in pleural pressure, resistance, and dynamic compliance. There are limitations to this method and therefore further investigation is needed into techniques that are less invasive and can continually monitor pulmonary function in horses. Respiratory inductance plethysmography may provide such a method.

The second chapter describes the experimental protocol and the results of continuously recorded respiratory movements made with respiratory inductance plethysmography technology in both control and RAO-affected animals.

In the third chapter I formulate conclusions based on the results described in the previous chapter, the implications for use of respiratory inductance plethysmography in future research, and how findings from respiratory inductance plethysmography may create new theories about the onset and progression of recurrent airway obstruction.

# CHAPTER 1

## LITERATURE REVIEW

### History of Recurrent Airway Obstruction

#### *Emphysema*

Recurrent airway obstruction (RAO) is an inflammatory, obstructive airway disease of the horse characterized by severe respiratory distress upon inhalation of organic dust [1]. Recurrent airway obstruction becomes clinically evident in susceptible middle-aged horses with clinical signs that include exaggerated nostril flare and abdominal effort. RAO is also known as heaves, emphysema, chronic obstructive pulmonary disease (COPD) and, in the United Kingdom, as broken wind. In layman's terms it is often described as an "asthma-like" disease in horses. Prior to explaining the pathology, etiology, and pulmonary function characteristics of RAO in depth, it is important to first understand the history behind this disease.

Knowledge of the mechanical properties of the lung assists scientists in the classification of lung diseases into two main categories: obstructive or restrictive. Obstructive diseases are simply defined as an obstruction of the airways in the lungs. This is documented by an increase in airway resistance during normal breathing. The resistance increases because of either an increase in secretions within the airways, bronchoconstriction, airway wall thickening, or destruction of the surrounding structures that support the airways. All of these forms of obstruction limit airflow into and out of the lungs, therefore decreasing gas exchange between the alveoli and the circulatory system. In people, examples of this type of disease include emphysema, chronic bronchitis, and asthma. Restrictive diseases cause a restriction in the expansion of the lung but the resistance within the airways does not increase. Examples include interstitial pulmonary fibrosis and pneumonia. Although the two classifications are distinct, both

can occur within the same patient. In this thesis, the topics discussed will solely be obstructive diseases.

In the 1930s the second leading causes of death in humans were pneumonia and influenza [2] how can two different disease be the second leading cause of death?. These diseases are restrictive diseases but the statistics encouraged investigation into human pulmonary pathology and eventually drove the search for suitable animal models of human lung diseases. In 1961, Mclaughlin, Tyler, and Canada identified the horse as the most similar to humans in subgross lung anatomy and also pointed out that the horse naturally expresses a disease, then termed emphysema, that has a similar counterpart in humans [3]. According to the NTA Bulletin as quoted by Gillespie and Tyler in 1969, the incidence of death due to emphysema rose to 82% during the years 1961-1965 [4]. Clinical symptoms and signs of emphysema in people include chronic cough, hyperinflation of the chest, abnormal breathing sounds either at rest or after exercise, and hyper-resonant percussion sounds upon auscultation [5], [6]. Radiographs revealed that, in patients in the supine position and suffering from emphysema, the position of the diaphragm is more inferior in healthy individuals. Therefore, the diagnostic criteria for emphysema were based upon a history of chronic cough and shortness of breath and the position of the diaphragm [5]. Unfortunately with this broad definition of emphysema, research protocols commonly included not only subjects who suffered from emphysema, but also those with bronchitis, and asthma. This led to a major misunderstanding that chronic cough originated from bronchitis and asthma and these conditions then developed into emphysema [6], [7]. These three conditions, currently known to be largely separate diseases, were considered to be just one disease.

The same terminology was transferred to the equine literature so that the common disease now termed recurrent airway obstruction was termed emphysema, and multiple hypotheses were proposed as to its cause. For example, previous upper airway infection was proposed to develop into chronic bronchitis, which further progressed to emphysema. According to McLean [8], discussing human disease, this theory included a three-stage process from bronchitis/bronchiolitis to emphysema. The first stage, bronchiolar obstruction, led to trapping of air within the alveoli (the second stage), and this hyperinflation of the gas exchange region caused the third process, which was disruption of the alveolar septa, resulting in the deformation of the bronchial wall as discovered at necropsy. Another unknown component was the effect of exposure to air pollutants within the stable environment [4]. The question became "could air pollutants increase susceptibility to an infection that would begin the cycle of bronchitis leading to emphysema?" Other researchers noted exacerbation of clinical signs due to an extended diet of hay, but upon removal of this hay the clinical signs were reversed [9]. These observations prompted the definition of structural and functional emphysema. Structural emphysema was defined as rupture of the alveolar walls, whereas functional emphysema, which, because it was due to peripheral airway obstruction, was potentially reversible, resulting in normal alveoli post mortem [10]. It was speculated therefore that one episode of heaves could occur without damage, but subsequent occurrences would lead to emphysema [9]. Others argued that the use of "functional" emphysema was improper because the term "emphysema" was, by definition, evidence of irreversible pathology upon necropsy [11]. The reversibility of clinical signs of heaves following removal of hay from the diet reported by Lowell [9] had been known since at least the 17$^{th}$ century and so the possibility of an allergic origin for RAO lingered. In order to investigate the possibility of allergy, Eyre [12] compared the results of skin tests in horses that

presented with clinical signs of “equine pulmonary emphysema” and a control group. Common moulds, plant pollen, and fungal antigens were tested and there were no significant differences between groups. It was concluded that skin testing could not solely be relied upon to determine a diagnosis. However, the authors noted once again the remarkable reversibility in clinical signs when the hay was removed from the diet and therefore concluded that the mechanism of heaves may still involve a mold allergy.

Gillespie and coauthors were the first to describe methods to measure pulmonary function in the emphysematous and control horses. The results indicated increases in intrathoracic pressure and in airway resistance at the end of expiration in diseased horses [13]. These findings are more characteristic of human asthma than of emphysema because, in the latter, airway resistance increases due to the smaller elastic recoil of the lung. In horses, Hermann Sasse was the first to coin the term chronic obstructive pulmonary disease (COPD) [14] based on the differences found in pulmonary function between control and diseased animals.

### *Chronic Obstructive Pulmonary Disease*

In humans, chronic obstructive pulmonary disease (COPD) is an umbrella term that includes both emphysema and chronic bronchitis. It is difficult to clearly diagnose the two as separate diseases prior to autopsy. The definition of emphysema is anatomic: it is a breakdown and loss of the alveolar septa. By contrast, chronic bronchitis is diagnosed based on clinical presentation of increased mucus production coupled with airway obstruction. The two diseases can occur simultaneously and therefore COPD was divided into two categorical types that represent different pathophysiology: type A and type B. Type A is characterized by shortness of breath, presence or absence of cough, and low mucus production. Radiographs reveal a flat

diaphragm, overinflation of the lungs, and narrowing of the pulmonary vessels. Type B is more severe in clinical presentation. A patient will have a history of chronic cough with acute exacerbations of sputum causing shortness of breath. Radiographs indicate congested lung fields and cardiac enlargement. Both types of COPD cause changes of the mechanical properties of the lung, which include an increase in resistance and reduction in ventilatory capacity, accompanied by hypoxemia [15].

As stated above, in the early 1970s chronic obstructive pulmonary disease (COPD) became the new term for heaves. Swiss professor Heinz Gerber argued that solely using the term emphysema to define heaves was inaccurate because it implied that heaves was irreversible, which it is not: based on the improved clinical signs after the susceptible animal has been removed from the stimulus show that heaves is reversible. Gerber examined fifty-two horses upon necropsy and noted the presence of chronic bronchitis in all cases. Emphysema was also noted in twenty-one out of the fifty-two cases. The histological findings in this group of horses were interpreted to indicate a potential for two types of obstructive diseases with an unknown origin. The author inferred that changes in pulmonary pathology could be due to infectious agents, allergens, or breed- and age-related factors [16]. These conclusions sparked further research into the identification of standard criteria necessary to diagnose COPD in horses as well as the appropriate definition of the clinical syndrome of heaves.

The observed double expiratory effort during normal respiration is the traditional characteristic feature of heaves. As more cases were presented, the main goal was to categorize all of the various clinical signs. Several studies investigated the differences between control and heaves-affected horses using both pulmonary function tests and radiographic evidence to determine the associations between structural and functional changes noted in the lungs

[16],[17]. It was deemed that a decrease in arterial oxygen level ($PaO_2$) and an increase in pleural pressure (Δ Ppl max) were indicators of disease severity. A history of poor performance, chronic cough, double expiratory effort, and an increase in breathing sounds were all noted as being specific to horses with heaves as compared to normal horses [17],[18]. Even with these advancements in the criteria used to diagnose the disease, there were still variations in the pathology described in clinical cases. This included emphysema, allergic asthma, and a disease similar to farmer's lung in man [17]. First, bronchospasm was investigated by Murphy and colleagues [19]. This group looked at the differences in pulmonary function, for example resistance and intrathoracic pressure changes, between affected and control horses after inhalation of three bronchodilators. It was shown that after the drugs were administered, the overall respiratory function improved. The most noted differences were decreases in the intrathoracic pressures and respiratory rates in affected animals. No changes were observed in control horses. The authors concluded that due to the decrease in the resistance within the airways of the affected group, proven by a decrease in the variables mentioned previously, bronchospasm represents a component responsible for the pathogenesis in heaves [19]. These findings paved the way for further research into the response of affected airways with inhalation of various agents such as histamine, common moulds, and a bacterium (*Sacharopolyspora rectivirgula* a.k.a. *Micropolyspora faenii*). Challenges with moulds and *S. rectivirgula* resulted in a loss of airway function in affected animals but not to the same extent of loss caused by a natural challenge. It was concluded therefore, that there is not just one agent responsible for heaves [20],[21],[22],[23]. Secondly, the long-recognized observation that clinical signs are reversed after the removal of hay and straw from the environment was further investigated by McPherson and Thomson [24]. They demonstrated that affected horses in remission did not

functionally differ from control horses, which therefore provided evidence that the disease is reversible and that “emphysema” was no longer an appropriate term for the disease. Throughout the 1990s, various terms were being used interchangeably to describe the pathology responsible for the disease but it was not until 2001 that two other pathological entities (allergic asthma and farmer’s lung) became the focus of investigation.

***Recurrent Airway Obstruction***

The term recurrent airway obstruction (RAO) was introduced by Derksen et al. [25] to describe clinical heaves and the exaggerated yet reversible bronchoconstrictor response to a variety of stimuli. The latter (not sure which items in previous sentence are referred to by “latter”) were more characteristic of human asthma and other chronic obstructive lung diseases. In 2001 at the International Workshop on Equine Chronic Airway Disease, COPD was no longer deemed the appropriate term to define heaves, because it denotes a largely irreversible disease that is associated with smoking in humans. The definition of recurrent airway obstruction (RAO) became “an inflammatory, obstructive airway disease caused by inhalation of organic dusts that becomes clinically evident in susceptible middle aged horses” [1]. RAO is associated with impaired pulmonary function, cholinergic bronchospasm, and an increase in neutrophilic inflammation and mucus accumulation in the airways [26],[27]. Bronchoalveolar lavage indicates a more than a 20% increase in neutrophils and a decrease in the percentages of macrophages and lymphocytes [28],[25],[29]. Environmental and genetic factors are known risks associated with not only the development of heaves, but also human asthma. Environmental factors include the housing conditions of the animal such as the type of bedding and nutrient supplementation. Horses that are fed hay, especially from round bales, have a higher percentage

of neutrophils within the airway in comparison to those that are kept on pasture [30]. When housed animals are fed hay on straw bedding a significant decrease in pulmonary function occurs in susceptible animals, but on shavings with a pelleted diet, pulmonary function can improve within three days [31]. Therefore, in order to induce the clinic signs of RAO, the standard challenged environment includes straw bedding and hay diet.

A genetic predisposition for RAO has been demonstrated by genetic epidemiological investigations in full and half-sibling groups [32],[33],[34]. In two Warmblood sires a linkage was made between an owner questionnaire designed to diagnose RAO and microsatellite markers in the chromosome 13q13 region in the family of sire 1 but not of sire 2. These results suggested a genetic background with locus heterogeneity for RAO [35]. To further expand on the marker found in sire 1, Swinburne et al. [36] performed a whole-genome scan on RAO-affected horses to determine the chromosome regions in which genetic variants are located. The authors found that two chromosome regions (ECA13 and ECA15) are significantly associated with RAO. Several interleukin genes are located within the two regions and specifically the IL4 receptor gene is found to play a role [37]. These genes are vital to the immune response and therefore may provide more information into the genetic factors associated with RAO. Overall the long-term management of RAO-affected horses involves keeping them on pasture. Generally this will keep them in clinical remission. Acute exacerbations of airway inflammation and obstruction are treated with pharmacological agents: corticosteroids to reduce inflammation and bronchodilators to reverse airway obstruction.

Current research is aimed at further investigating the pathophysiology responsible for the inflammation and obstruction that lead to the clinical signs; for example, reversibility of airway remodeling during remission, drug efficacy, genetic factors, environmental factors, and less

invasive diagnostics. It is estimated that 20% of the pleasure horse population is affected by lower airway inflammation [30]. The aging population of horses is increasing and therefore the prevalence of RAO may increase over time. Therefore, reducing the incidence of RAO and less severe forms of lower airway inflammation is important to the equine industry. In addition, RAO also serves as an animal model for human pulmonary research.

**Evaluating Disease**

The trigger associated with the onset of clinical exacerbations of RAO is inhalation of organic dusts [31], [38]. These agents may comprise species of bacteria and their products, including endotoxin, moulds, forage mites, and inorganic components [39], [40], [41]. To induce airway obstruction, RAO-susceptible horses are commonly placed into what is called a "challenge environment," which is usually a stall containing straw bedding and where the horse is fed poor-quality, dusty hay. Clinical exacerbations are evident by an exaggerated nostril flare and increased abdominal effort during breathing. To clinically score disease severity, these signs are each given numerical values ranging from 1 (normal) to 4 (maximal nostril flare on both inhalation and exhalation and severe abdominal effort during each breath) [42].

Pulmonary function tests provide a more quantitative method of evaluating disease severity that can be used in the standing unsedated horse [13], [43], [44], [45]. These require the placement of an esophageal balloon and use of a pneumotachograph attached to a facemask to measure changes in pleural pressure and air flow, respectively, in order to calculate pulmonary variables such as resistance and dynamic compliance. Horses are intubated with an esophageal balloon sealed over the end of a 240-cm-long polyethylene catheter (2.4 mm internal diameter, 4 mm external diameter) with five lateral holes drilled in the portion covered by the balloon. The balloon is passed via a nasogastric tube and positioned in the intrathoracic esophagus, caudal to

the heart base but cranial to the diaphragm. Pressure changes within the balloon are measured by a pressure transducer (calibrated against a water manometer). The maximal change in pleural pressure during each tidal volume (ΔPplmax) is calculated from the peak to base amplitude of the inspiratory pressure signal. A plastic facemask (Aeromask, Trudell Medical International) with a rubber seal is placed on the nose of the horse. A pneumotachograph is inserted into the facemask at the nose to measure air flow. Calibration of the pneumotachograph is done with a rotameter through which air is being blown. The values for ΔPpl and flow are transferred to a computer where software calculates tidal volume (the integral of flow from start to end of inspiration), dynamic compliance, and lung resistance. Dynamic compliance (L/cm $H_2O$) is calculated by ΔV/ΔP between the start and end of inspiration. Lung resistance (cm $H_2O$/L/s) is calculated from ΔP and ΔFlow occurring between two points corresponding to 75% of tidal volume on inspiration and expiration [45].

The main limitation to this procedure is its invasive nature, which means that these tests can be performed only in a laboratory setting with trained animals and experienced staff. The placement of the esophageal balloon and facemask are not well tolerated, even in trained horses, which can become non-compliant during repeated measurements. This limits the number of breaths that can be analyzed to a short interval. Consequently, little data has been collected on horses out in the field, and investigations have been limited to the severe, perhaps end-stage RAO-phenotype of horses that are donated to research laboratories. There is no information about less severe phenotypes, i.e., the precursors of RAO, because these horses do not have extreme clinical signs or measurable changes in pulmonary function. Therefore, it is important to investigate other methods that may be useful in determining subtle changes in airway function during the onset and progression of the disease.

**Respiratory Inductance Plethysmography**

As previously stated, standard pulmonary function tests in horses are limited in the number of consecutive breaths that can be measured due to the cumbersome nature of the procedure. The ability to measure respiratory movements on a 24-hour basis has the potential to allow investigation into the onset and progression of RAO and to aid in the investigation of future therapeutic targets for horses. Respiratory inductance plethysmography (RIP) was first introduced in 1977 for noninvasive ambulatory measurement of human respiratory movements [46]. The advantage of RIP is that it measures ventilation on a breath-by-breath basis. Volume and timing variables of respiration measured by RIP include tidal volume ($V_T$), frequency (f), minute ventilation ($V_e$), inspiratory duration ($T_i$), and expiratory duration ($T_e$). This is accomplished by measuring the changes in the cross-sectional area of both the thorax and abdomen. The plethysmography equipment consists of two cotton bands that contain a zig-zag copper wire. One band is placed on the thorax and the other on the abdomen. As alternating electrical current flows through the wire, a voltage is generated creating a magnetic field. During respiration the movement of both the thoracic and abdominal bands changes the shape of the magnetic field, which induces an opposing current that can be measured, which corresponds to changes in the cross-sectional area of the two compartments [47]. The sum of the changes in the two bands is an indication of the total volume of air being breathed into and out of the lung. The two separate signals from the bands are first sent telemetrically to a receiver that is attached to an analog to digital converter, and then to the computer software, which analyzes the two bands on a breathto-breath basis.

The following explanation of respiratory movements in the horse demonstrates why it is necessary to sum the changes in the cross-sectional area of both thorax and abdomen to accurately understand breathing in the horse. Horses have a biphasic breathing pattern, with active and passive components to both the inspiratory and the expiratory flow. In addition, rather than breathing from the mechanical equilibrium of the respiratory system, they breathe around the mechanical equilibrium of the respiratory system, which is known as functional residual capacity (FRC), [48]. At the end of exhalation, the lung volume is below FRC because the abdominal muscles are contracted and the viscera are compressing the diaphragm into the thorax. During the first phase of inhalation, the respiratory system passively returns to FRC due to the relaxation of the abdominal muscles, which allows the abdominal contents and diaphragm to displace caudally and the lungs to expand. Abdominal relaxation coupled with caudal displacement of the diaphragm and viscera by the expanding lung increases the cross-sectional area of the abdomen. The second phase of inhalation is active and is initiated by contraction of the diaphragm. This caudal displacement of the diaphragm further increases the abdominal cross section and also creates a negative pressure gradient between the atmosphere and the lungs, which allows air movement into the lungs. Concurrently, contraction of the external intercostal muscles stiffens the rib cage and pulls it forward and outward. The movement of the rib cage increases the cross-sectional area of the thorax. The sum of these changes in the thoracic and abdominal cross-sectional areas are an indication of the total volume of air breathed into the lung during inhalation. In the resting animal, the first phase of exhalation is the passive recoil of the lung back down toward the FRC. This causes inward movement of the rib cage and the cranial movement of the diaphragm resulting in changes of the cross-sectional area of the thorax. The second phase of exhalation is active due to the contraction of abdominal muscles forcing the lung

volume below FRC in order to prepare for the passive phase of inhalation. The contraction of the abdominal muscles results in a decrease in the cross-sectional area of the abdomen [49]. The sum of the two signals is an indication of the total airflow into and out of the lungs during exhalation. In summary, the basic mechanism of breathing allows the respiratory inductance plethysmography bands to monitor patterns of breathing.

Ventilatory variables measured by RIP are obtained by analysis of each individual breath. The sum of the displacement of the two bands provides the total respiratory displacement (RIPtd), which is an indicator of tidal volume, and the reciprocal of the total duration (Ttot) of each breath provides an instantaneous value of frequency (respiratory rate) based on each breath. Minute ventilation is then calculated as the product of tidal volume and respiratory rate. Calibration of the two bands to real-time air flow measured via a pneumotachograph allows the RIPtd to be expressed as tidal volume in liters. Calibration to real-time air flow is not necessary when just assessing timing variables of ventilation. A study of thirty-seven children that compared tidal breathing parameters such as the ratio of inspiratory time to the total time of each breath ($t_i/t_{tot}$) and respiratory frequency showed good agreement between simultaneouslymeasured noncalibrated RIP and by a facemask-pneumotachograph system [50].

The traditional plethysmographic measurements in human medicine involve placement of a mouthpiece and noseclip onto the patient. However, this method has been shown to significantly alter volume and timing variables of respiration in comparison to RIP. Use of RIP demonstrated that the mouthpiece and noseclip method specifically increases mean values of tidal volume, minute ventilation, inspiratory and expiratory time, and decrease respiratory frequency in healthy human patients [51].

**Breathing Pattern Variability**

The advantage to the RIP technology is that analyses can be performed on a breath-by-breath basis over long periods of time. This provides the means by which to examine breathing patterns during respiration by calculating measures of variability such as standard deviation and the coefficient of variation for each variable [52]. Analysis of the variability has demonstrated differences in breathing patterns between healthy people and those affected with obstructive airway diseases.

One study measured timing variables via respiratory inductance plethysmography in COPD and control patients [53]. The coefficient of variation in inspiratory time ($T\dot{i}$) and the ratio of inspiratory time and the total time of each breath ($t_i/t_{tot}$) were decreased in COPD patients but not in controls. These results indicate that changes in variability occur in an obstructive disease. This disease is similar to that of RAO and this type of analysis may prove beneficial in the investigation of airway diseases in horses.

There has been limited research on the use of RIP technology in animals. The earliest use was on sheep to compare the relative percent deviations of tidal volume as measured by the pneumotachograph-facemask system and RIP bands pre- and post-carbachol-induced bronchoconstriction. At both baseline and after carbachol administration, the tidal volume measured by the pneumotachograph and by RIP differed by only ± 6%. These results indicate that RIP provides an accurate measure of ventilation in this animal [54]. A similar type of study was not done in horses until 2001. Horses expressing clinical signs of recurrent airway obstruction were fitted with the bands as well as a pneumotachograph and facemask. Comparisons were made between the two methods, and RIP was determined to be a valid

measurement of relative changes in lung mechanics in horses [55]. The temporal relationships between thoracic and abdominal movements can be calculated by determination of the phase angle of the two bands. In RAO-affected horses, the rib cage leads ventilation during both inspiration and expiration, with the abdomen lagging [56].

## Conclusion

During severe exacerbations of recurrent airway obstruction, clinical signs and alterations in pleural pressure (ΔPplmax), lung resistance (RL), and dynamic compliance (Cdyn) can be determined. However, there are limited data available concerning the onset and progression of this disease. Due to the limitations of the pneumotachograph-facemask system it is crucial to utilize a noninvasive method in order to evaluate respiration continuously. Respiratory inductance plethysmography (RIP) provides the ability to monitor breathing on a breath-by-breath basis.

In the next chapter I will explain how we tested the hypothesis that RIP will detect differences between control and RAO-affected horses and the implications for these results.

# CHAPTER 2

# TELEMETRIC MONITORING REVEALS REDUCED BREATHING PATTERN VARIABILITY IN HEAVES-AFFECTED HORSES

## Introduction

Recurrent airway obstruction (RAO) is an airway disease that becomes clinically evident in susceptible middle-aged horses [1]. The obstruction by bronchospasm and excess mucoid secretions is a consequence of inflammation that occurs within a few hours of inhalation of organic dust. To understand the sequence of events in airway obstruction, it will be necessary to closely relate airway dysfunction to the accompanying inflammatory events. For the past four decades changes in the severity of airway obstruction in RAO have been assessed by a variety of lung function tests that are slightly invasive because they require use of a facemask pneumotachograph system with or without an esophageal balloon [13],[57]. Because of the invasiveness of the equipment, only snapshots of respiratory function can be obtained and only in cooperative horses. This makes it difficult to continually study respiration during the onset and progression of the disease.

Clinicians evaluate the respiratory system by observing the breathing pattern, which incorporates the effort, the respiratory rate, and the timing of inspiration and expiration. These clinical signs are obvious in cases of severe RAO in which the abdominal effort is increased, expiration is prolonged, and there is little variability in pattern among breaths. By contrast, in healthy horses, breathing can be difficult to discern and the pattern is quite variable as horses sniff and sigh in among more regular breaths. Horses with less severe RAO can be difficult to distinguish from control animals even though the biphasic breathing pattern characteristic of

control horses is lost and peak inspiratory flow is increased [45]. In order to follow these changes during the onset of RAO, there is a need to evaluate a noninvasive method that can continually monitor breathing pattern. Respiratory inductance plethysmography (RIP) provides such a tool. For this reason, we hypothesized that RIP would detect differences between RAO-affected and control horses within a few hours of stabling.

Respiratory inductance plethysmography is not invasive, does not need a facemask, and when used with telemetry, continually records respiratory movements without interfering with the horse's behavior. RIP measures changes in the cross-sectional area of the thorax and abdomen by means of two cotton bands containing a coiled metal wire through which an alternating electrical current passes, thus creating a magnetic field. The respiratory movements of both the thoracic and abdominal compartments change the shape of the magnetic field and induce a measurable opposing electric current proportional to the change in the cross-sectional area [58]. The sum of the output of the two bands provides the total respiratory displacement (RIPtd), which is an indicator of tidal volume, and the reciprocal of the total duration ($T_{tot}$) of each breath provides an instantaneous value of frequency (respiratory rate, RIPf) for each breath.

In human medicine, RIP has been used to assess respiration in young children who do not tolerate the placement of the facemask-pneumotachograph system. A study of thirty-seven children that compared tidal breathing parameters such as the ratio of inspiratory time to the total time of each breath ($t_i/t_{tot}$) and respiratory frequency showed good agreement between simultaneously measured noncalibrated RIP and by a facemask-pneumotachograph system [50]. A similar comparison indicated that RIP accurately monitors breathing pattern in sheep both with and without provoked bronchospasm [54]. In horses, simultaneous recordings of breathing using

RIP and a pneumotachograph have been made for approximately 50 breaths during an acute exacerbation of heaves [56], but no one has monitored respiration continually in either healthy or RAO-affected horses throughout the day.

In this study, therefore, we used RIP to test our hypothesis that RIP would detect differences between RAO-affected and control horses within a few hours of stabling. The investigation revealed that significant changes in breathing pattern develop within eight hours of stabling in RAO-affected horses.

## Materials and Methods

### *Introduction*

The investigation used two protocols. In protocol 1, control horses were used to determine the optimal abdominal RIP band placement. In protocol 2, RIP was used to investigate differences in breathing pattern between control and RAO-affected horses during the onset of airway obstruction induced by stabling. During this time, pulmonary resistance (Rl), dynamic compliance (Cdyn), and maximal change in pleural pressure (ΔPplmax) were measured daily to quantify the severity of airway obstruction.

### *Animals*

The All-University Committee for Animal Use and Care of Michigan State University approved the protocols. Protocol 1 included twelve horses with no history or clinical signs of respiratory disease (11 mares and 1 gelding), 5-19 years old (mean ± SD, 11.9 ± 4.3 years) with

weights ranging from 445 to545 kg (mean ± SD, 489 ± 35kg). In protocol 2, two horse groups were used: control and RAO-affected (n = 14: control and RAO-affected were evaluated in 7 pairs). The affected group had a previous diagnosis of RAO (4 mares and 3 geldings), was 13-32 years old (mean ± SD, 21 ± 6.4 years) with weights ranging from 475and 545 kg (mean ± SD, 503 ± 36 kg). The control group included 6 mares and 1 gelding, 5-19 years old (mean ± SD, 9.1 ± 5.0 years) with weights ranging from 445 to 545kg (mean ± SD, 486 ± 33 kg). All horses were maintained on pasture and supplemented with a pelleted diet except during periods of stabling and exposure to hay and straw bedding in order to induce acute exacerbations of RAO [20].

***Respiratory Inductance Plethysmography***

The two RIP bands[a] were connected to a transmitter box (2 cm × 5 cm × 4 cm) that telemetrically sent the RIP signal to a receiver connected to an analog-to-digital (A-D) converter. The transmitter box was attached to a breast collar and the mane so that it hung on the left side of the neck adjacent to the cranial border of the scapula.

Computer software[a] analyzed the RIP output from the two bands in real time on a breath-to-breath basis. For the purposes of the present investigation, the variables of interest were instantaneous respiratory frequency (RIPf), and abdominal (RIPabd), thoracic (RIPth), and total inspiratory displacement (RIPtd). The RIPf was calculated as the inverse of breath duration, and displacements were calculated from the change in the cross-sectional area of the thoracic and abdominal compartment. The latter were summed to derive RIPtd. No attempt was made to correlate displacements with independently measured changes in absolute lung volume.

***Pulmonary Function Tests***

Horses were intubated with an esophageal balloon sealed over the end of a 240-cm-long polypropylene catheter (2.4 mm internal diameter, 4 mm external diameter) with five lateral holes drilled in the portion covered by the balloon. The balloon was passed via a nasogastric tube and positioned in the intrathoracic esophagus, caudal to the heart base but cranial to the diaphragm. Pressure changes within the balloon were measured by a pressure transducer[a] calibrated daily against a water manometer [43]. A plastic facemask[b] was placed over the nose and sealed around the face by means of a rubber cuff and electrical tape. A pneumotachograph attached to a pressure transducer was inserted into the front of the facemask for measurement of airflow. Calibration was performed daily using a rotameter flow meter. The transducer signals for esophageal pressure and flow were passed through an A-D converter and amplifier and then analyzed with a software program[a]. The outputs recorded were change in pleural pressure ($\Delta$Ppl) during inspiration, pulmonary resistance (RL) calculated by the isovolume method at 75% tidal volume, and dynamic compliance (Cdyn). Because the software could not calculate maximal change in pleural pressure ($\Delta$Pplmax), it was manually calculated from the printed output of the esophageal pressure signal. For each variable, data were collected from 20 breaths greater than 1 liter.

***Clinical Score***

Clinical signs were scored daily by the same individual. The score was derived by summing the degree of nasal flaring and abdominal effort (each scored 1-4) [59].

***Experimental Design and Data Analysis***

*Protocol 1 – Determination of optimal abdominal RIP band placement*

Anatomically, intercostal space (ICS) 6 encompasses only the thorax while more caudal intercostal spaces surround increasing amounts of abdomen. This experiment compared the RIP output of the thoracic band fixed at the $6^{th}$ intercostal space as the abdominal band was randomly moved among intercostal spaces 9, 11, 13, 15, and 17 and over the abdomen just caudal to the $18^{th}$ rib [56]. At each location RIP output was recorded for one minute. Comparisons of RIP output between the fixed thoracic band and the abdominal band placed at the various intercostal spaces was by $ANOVA^{c}$ (random horse, fixed intercostal spaces) and Tukey's HSD test with $P < 0.05$.

*Protocol 2 – Comparison of RIP output in RAO-affected and control horses*

Each pair of horses (control and RAO-affected) was brought in from pasture for baseline measurements of both clinical score and pulmonary function. If the clinical score and average ΔPplmax met the entrance criteria of ≤ 4 out of 8 and < 10 cm $H_20$, respectively, the RIP bands were then placed and secured with loosely applied horizontal mattress sutures that passed over but allowed free stretching of the bands. RIP data were recorded throughout the day except for the approximately one hour around 9 am when lung function was measured. Throughout the protocol, horses were bedded on straw and fed poor-quality hay. Each pair was stabled for seven days unless the RAO-affected horse developed a clinical score of 8/8 that persisted for two consecutive days.

The daily RIP output was divided into periods 1 through 6, which were equivalent to 10.00–14.00 h, 14.00–18.00 h, 18.00–22.00 h, 22.00–02.00 h, 02.00–06.00 h, and 06.00–09.00 h, respectively. Each period contained approximately 4,000 breaths. The means and standard deviations of RIPf and RIPtd were calculated for each four-hour period. Both data on lung function (ΔPpl, ΔPplmax, RL and Cdyn) and RIP output (RIPf and RIPtd) were analyzed using a split plot repeated measures ANOVA[c] ($P < 0.05$). Factors that could influence these response variables for lung function were horse, disease (control and RAO), and the repeated factors of day (0, 1, 2 days). Factors that could influence these response variables for RIP analysis were the same as lung function, with an additional repeat factor of period (6 per day). Pearson's correlation was used to examine the relationship between lung function and RIP variables.

## Results

### *Protocol 1 – Determination of optimal abdominal RIP band placement*

The abdominal band displacement increased from intercostal space (ICS) 9 to 17, becoming the largest at ICS 17 (Figure 1). Displacement in ICS 11 through 17 was significantly different from that at ICS 9, but only ICS 17 displacement was different from that at both ICS 9 and 11. Additionally spaces 11-17 all differed significantly from the thoracic band at ICS 6. When the abdominal band was placed caudal to the 18$^{th}$ rib, its displacement did not differ from that in ICS 17, but the variability of displacement (1.4 ± 1.1, mean ± S.D.) was greater. Because ICS 17 had the largest displacement, differed significantly from ICS 6, and was less variable than caudal to the 18$^{th}$ rib, it was selected for the abdominal RIP band. Furthermore, radiographs

confirmed that ICS 6 was totally over the thorax and ICS 17 was caudal to the diaphragm (Figure 2).

***Protocol 2 – Comparison of RIP output in RAO-affected and control horses***

*Pulmonary function*

Stabling induced airway obstruction in the RAO-affected horses at varying rates. Four horses completed the 7-day exposure. The remaining four had to be removed from the protocol after 4 days due to the severity of their obstruction. For this reason, we statistically analyzed 4 days of data as follows: baseline, baseline + 1 day, final - 1 day, and final day of stabling. The ΔPplmax of RAO-affected horses increased during stabling and differed significantly between groups on the final-1 and final day of stabling (P = 0.007 and 0.004, respectively) (Figure 3). Likewise, the Cdyn significantly decreased in the RAO-affected group and was significantly different from the control group on both the final -1 day and the final (P = 0004 and P = <0.001, respectively) day of stabling. Although it was decreased on the final-1 day and final day in the RAO-affected group, RL only differed significantly from the control group on the final day (P = 0.012). Stabling did not significantly alter the ΔPplmax, RL, or Cdyn in the control group.

*Respiratory Inductance Plethysmography*

Although neither RIPtd nor RIPf differed between groups or changed during stabling (Figure 4) there were considerable differences in breathing patterns between RAO-affected and control horses (Figure 5). In control horses the amplitude and duration of each breath varied over time: short and quick breaths were often followed by bigger, less frequent breaths. In contrast, the RAO-affected horses expressed a repetitive pattern in both amplitude and duration with little

variability among breaths. This variability was evaluated by calculating the standard deviations of RIPtd and RIPf (SDRIPtd and SDRIPf, respectively) for the six time blocks in each day. In the first 24 hours of stabling SDRIPtd and SDRIPf did not change in control horses but decreased in the RAO-affected animals. The two groups differed significantly by 8 hours after stabling (Figure 6). These significant differences between groups persisted during the final 24 hours of stabling.

Examination of Figure 7 suggests a circadian rhythm to both SDRIPtd and SDRIPf. For this reason we used the split-plot ANOVA to examine the main effect of time periods during the day. The statistical results were the same for both of these variables. In control horses, SDRIPDtd and SDRIPf were significantly greater at period 6 (06.00–09.00 h) than at all other periods ($P < 0.05$). In the RAO-affected horses, the circadian variation was more obvious with a statistically significant nadir at periods 3 and 4 (18.00–02.00 h). At all times except period 1 (10.00–14.00 h), the SDRIPDtd and SDRIPf were significantly less in RAO-affected than control horses.

To determine the relationship between both SDRIPDtd and SDRIPf and pulmonary function variables, we performed correlation analysis using RIP output at period 2 of each day. Period 2 was selected because it was the point at which significant group differences occurred following stabling. Correlation between lung function and SDRIPDtd and SDRIPf was initially poor, but over the final 24 hours, lung function and SDRIPDtd and SDRIPf were highly correlated (Table 1). Generally both SDRIPDtd and SDRIPf were better correlated with $\Delta$Pplmax than with RL or Cdyn.

**Discussion**

To our knowledge this is the first description of continuously monitored breathing patterns in unrestrained horses for more than 24 hours. The cumbersome nature of conventional pulmonary function tests limits the ability to analyze sequential breathing patterns over an extended period of time. Therefore a less invasive method, RIP, was investigated in this study. This revealed that significant decreases in breathing pattern variability develop within 8 hours of stabling in RAO-affected horses, which is well before significant changes in ΔPplmax, RL, or Cdyn.

Respiratory inductance plethysmography measures changes in the cross-sectional area of the thorax and abdomen. During inhalation, the cross-sectional area of the rib cage increases; in addition, contraction of the diaphragm displaces the abdominal contents, resulting in an increase in the cross-sectional area of the abdomen. For this reason, it is necessary to sum the displacements of the thoracic and abdominal compartments to provide an indication of the total volume inhaled. The advantage of RIP is that it is relatively non-invasive and, when coupled with telemetry, allows monitoring of breathing over long periods of time. Relating RIP band data to absolute changes in volume measured at the nares can be problematic, especially in the presence of airway obstruction. This is because of gas trapping within the lung, which results in gas compression and phase differences between RIP and volumes measured at the external nares [56]. Because we were interested solely in using RIP as a simple indicator of the presence of lung disease, and not in phase relationships, RIP bands were not calibrated to absolute volumes and therefore total displacement was only reported as relative liters. Traditionally, respiratory frequency is determined by counting breaths per minute, but in the present study, the frequency was calculated breath-by-breath as the reciprocal of the duration of each breath. Calculating frequency from each breath provided greater power to examine variability.

The main objective of protocol 1 was to determine optimal band placement for measurement of the thoracic and abdominal components of ventilation. One RIP band was maintained at ICS 6 while the second was randomly placed over the more caudal odd-numbered spaces. The anatomy of the diaphragm and rib cage provides the basis for understanding the effect of ICS space on RIP displacement. Because the cranial ribs form part of the pectoral girdle, their respiratory movements are limited. The asternal and floating ribs have more freedom of movement and therefore contribute more to the total respiratory displacement. ICS 6 was chosen for the thoracic band because it encloses only the thoracic contents and is between two sternal ribs. As the abdominal band was placed more caudally, two factors contributed to the increasing RIP displacement: the movement of the asternal ribs and the outward movement of the abdominal wall that results from caudal displacement of the diaphragm.

Significant differences between the thoracic band at ICS 6 and the abdominal band did not occur until ICS 11 and the mean displacements for ICS 13-17 were not significantly different from one another. Because, ICS 17 was totally over the abdomen and had the greatest displacement, it was selected as the optimal location to measure abdominal excursions. Hoffman et al. [56] placed the abdominal RIP band behind the $18^{th}$ rib. In our study, data from this location were highly variable and only significantly different from ICS 6 and 9.

During the environmental challenge we evaluated RIP and pulmonary function variables in normal and RAO-affected horses. Pulmonary function tests indicated the onset of airway obstruction in the RAO-affected but not in the control horses. The ΔPplmax and RL increased and Cdyn decreased in the RAO-affected horses and became significantly different from controls on the final two days of stabling.

The RIP output of total displacement and frequency did not differ between groups or throughout stabling. Previous studies using pulmonary function tests have shown that tidal volume is unaffected by stabling in both control and RAO-affected animals [45]. Previous reports of changes in frequency with stabling are conflicting [45], [60].

Even though breathing frequency did not differ between groups nor change with stabling, further observation of the 24-hour RIP traces indicated a considerably different pattern of breathing between the control and RAO-affected animals (Figure 5). In order to quantitatively express this difference, we calculated the standard deviation of both total displacement and frequency (SDRIPtd and SDRIPf, respectively). In contrast to mean RIPtd and RIPf, both SDRIPtd and SDRIPf of RAO-affected horses significantly decreased by eight hours after stabling. These values were unchanged in the control animals. This significant difference between RAO-affected and control animals persisted throughout stabling. The only way to explain a decreased standard deviation of frequency and total displacement in the absence of a change in mean values is that breathing pattern of RAO-affected animals becomes less variable as disease becomes more severe. As breathing approaches a regular wave form, each breath approaches the same RIP displacement (tidal volume) and lasts for the same duration (Figure 5).

This loss in variability has to involve respiratory control mechanisms, the main goal of which is maintenance of gas exchange. For the latter to occur, the brain receives sensory input from various sources and appropriately adjusts the rate and depth of breathing. These sources include: a) chemoreceptors, b) airway and intra-pulmonary receptors, and c) muscle stretch receptors. Peripheral chemoreceptors monitor changes in $PaO_2$, $PaCO_2$, and pH. Pulmonary and airway receptors innervated by vagal afferent nerves include stretch, irritant, and C fiber receptors that can activate bronchoconstriction, initiate cough, and change rate and depth of

breathing. Intercostal muscle stretch receptors adjust the force of contraction in order to maintain ventilation in the face of airway obstruction and other changes in the mechanical properties of the lung.

In RAO it is possible that all of the above provide inputs to respiratory control because inflammation leads to airway obstruction, which causes ventilation-perfusion mismatching, resulting in hypoxaemia [61]. It seems unlikely, however, that decreased $PaO_2$ is an important regulator of the breathing pattern because administration of oxygen in a dose-dependent manner to increase $PaO_2$ to 100 mmHg and above has no effect on respiratory rate [62]. In contrast, vagal afferents may play an important role in regulating breathing in RAO-affected horses. When using ovalbumin-sensitized and -challenged ponies to model RAO, Derksen et al.[63] observed that vagal blockade decreased respiratory rate. This is in line with the considerable evidence for the involvement of vagal afferent fibers, such as pulmonary C-fibers and rapidly adapting stretch receptors, as contributors to dyspnea in people [64]. The sensitivity of these pulmonary afferents to stimuli increases in the presence of airway inflammation and these changes can last well beyond the period of acute inflammation [65]. Therefore, we propose that the recurrent bouts of inflammation characteristic of RAO up-regulate pulmonary afferent sensory function, thus causing alterations in breathing patterns. Interestingly, in RAO, neutrophil influx into the lung occurs within six hours after stabling [66], which coincides with the onset of decreased variability of breathing, i.e., significant decreases in SDRIPf and SDRIPtd. Absence of decreased variability in controls is presumably because their reported transient inflammation that occurs during stabling is of much lesser magnitude than in the RAO-affected animals [67].

A circadian rhythm in the breathing variability was especially evident in the RAO-affected group with the significant nadir of SDRIPf and SDRIPtd occurring in the evening and early night (18.00–02.00 h). This circadian variation in the breathing patterns coincides with the diurnal variation in cortisol levels. In normal horses, serum cortisol concentrations peak during the morning (06.00–09.00 h) and reach a trough in the evening hours (19.00–23.00 h) [68],[69],[70],[71]. Due to the anti-inflammatory properties of cortisol, it is possible that as the cortisol levels decrease, pulmonary inflammation increases. The latter increases activity in pulmonary afferent nerves and alters the breathing pattern of RAO-affected horses. In controls, the circadian rhythm likely was minimal, most likely because the airway inflammation was minimal. Confirmation of our hypothesis will require concurrent measurement of breathing variability, inflammatory markers, and serum cortisol.

Comparisons were made between SDRIPtd and SDRIPf and pulmonary function test variables ΔPplmax , Rl, and Cdyn to determine the relationship between the two measurements. Although SDRIPtd and SDRIPf became significantly less in RAO-affected than control horses within eight hours after stabling began, significant differences in pulmonary function between the two groups did not occur until the final two days of stabling. This explains the poor correlation between breathing variability and lung function during the early stages of the disease. However, as the severity of airway obstruction increased, SDRIPtd and SDRIPf and pulmonary function variables became highly correlated, especially ΔPplmax. Overall, these observations indicate that SDRIPtd and SDRIPf are early indicators of RAO-exacerbations.

In summary, telemetric respiratory inductance plethysmography is a non-invasive technique that can measure variations in breathing patterns of normal and RAO-affected horses over long periods of time. This allows monitoring of horses in a challenge environment during

the onset and progression of disease. If, as we postulate, airway inflammation alters sensory nerve activity resulting in the early changes in breathing patterns, RIP will provide one tool to gain new insights into inflammatory mechanisms occurring during the onset of the disease.

# CHAPTER 3

## FUTURE PERSPECTIVES

As shown in the results of this investigation, RIP is a useful technique to compare changes in breathing patterns between control and RAO-affected animals. The novelty of this equipment is its ability to non-invasively measure the breathing pattern of horses over a 24-hour period of time. This technique could further be utilized during experiments not only in other species but in other disease models. For example, it has been shown that heart rate variability is associated with inhalation of particulate matter in hypertensive rats [72]. Respiratory inductance plethysmography may be beneficial for minute-by-minute comparisons of cardiac and respiratory function in the presence of air pollution. The RIP technique clearly is not limited to just RAO. Taking it a step further, RIP may prove beneficial in a clinical setting to monitor the progress of respiratory diseases in foals. Due to the volatile nature of foals, invasive procedures that measure respiratory mechanics are rarely performed. Respiratory inductance plethysmography might aid in diagnosing pulmonary issues without altering the natural environment of the foal. One important disease in which this could be useful is *R. equi* pneumonia, which has an insidious onset so that foals are rarely diagnosed until the lung is severely injured [73]. Continual RIP monitoring of at risk foals might provide a useful early clue to the presence of disease. Other species that are difficult to handle, such as alpacas and llamas, have been investigated in a research setting [74],[75], but now RIP could be used for clinical assessment as well. However, even though advancements can be made with RIP, limitations do arise no matter the level of invasiveness when investigating the pathophysiology of disease. This is due to the “phenotyping uncertainty principle” which states that the more one precisely wishes to characterize some aspect of physiology the less relevant to normal functioning are the conditions under which

characterization must be made [76]. One can interpret this statement to mean that there is a continuous line with the least invasive procedures on one end and the most invasive and precise on the opposite end. No method is optimal but somewhere in the middle is the appropriate technique to use and it is dependent on the question at hand.

An obvious continuation of the results of my investigation would be the application of RIP in field studies. Field studies allow for a greater population of horses to be investigated and might provide insight into the various phenotypes of severity in RAO. In a research setting it is difficult to account for all the various factors in a client horse's environment. In order to consult on the management of these various factors, one must experience the environment first hand. The results of my investigation indicate that significant differences between RAO-affected and control horses were found when the variability of breathing frequency in the RAO-affected animals was at or below 10 breaths/min. Using this value as a cutoff limit for expression of RAO might provide an efficient means to investigate drug efficacy in a larger population of horses.

As previously stated, investigation is needed into the mechanisms responsible for the decrease in variability. The first question that needs to be answered is does this decrease in variability occur with all inflammatory diseases in the horse or is it specific to the pathophysiology of RAO. A simple way to investigate this question would be to analyze breathing patterns in horses with pneumonia. To go further, does the decrease in variability occur with just systemic inflammation or strictly with pulmonary inflammation? These questions can be answered, however the model we are most interested in is RAO. The two main components in the pathophysiology of RAO are inflammation and obstruction. Afferent and efferent signals to the brain are responsible for changes in breathing pattern. The afferent signals originate from the lungs and carotid bodies that then send signals to the brain via the vagus and glossopharyngeal

nerves, respectively. The brain then sends the efferent signals via the phrenic and intercostal nerves to the respiratory muscles in order to change respiration. In RAO, the inflammation that precedes the obstruction activates C-fibers and increases the sensitivity of irritant receptors [64]. Bronchoconstriction narrows the airways, which could alter the sensitivity of the pulmonary stretch receptors located in the walls of the larger bronchi. These receptors are responsible for the termination of inhalation and a change in their sensitivity could change the relationship between tidal volume and breathing frequency. Investigations need to be done to determine which of these signaling pathways are responsible for the decrease in variability. These investigations could begin by administering target drugs that solely induce bronchonconstriction or inflammation in healthy animals or by using bronchodilators or anti-inflammatory treatments in RAO-affected animals. A model for strictly bronchoconstriction is administration of methylcholine. A model for solely inducing inflammation is administration of dilute acetic acid [77]. Creating a protocol that analyzes the effects on breathing pattern with these two different agents might provide insight into the mechanism responsible for the decrease in variability in RAO-affected horses.

In summary many different protocols utilizing RIP can be conducted not only in horses but other species and various disease models. Continuing the use of this technology in the investigation of RAO will provide more information into the onset and progression of the disease.

# APPENDIX

| Correlation Coefficient Values ($r^2$) for Total Displacement | | | |
|---|---|---|---|
| | Baseline | Final -1 | Final |
| Δ Pplmax | 0.56 | 0.78 | 0.9 |
| Resistance | 0.73 | 0.35 | 0.75 |
| Dynamic Compliance | 0.51 | 0.64 | 0.58 |

| Correlation Coefficient Values ($r^2$) for Frequency | | | |
|---|---|---|---|
| | Baseline | Final -1 | Final |
| Δ Pplmax | 0.63 | 0.81 | 0.82 |
| Resistance | 0.51 | 0.32 | 0.44 |
| Dynamic Compliance | 0.82 | 0.73 | 0.56 |

**Table 1:** Correlation coefficient results between RIP output (SDRIPf and SDRIPtd) and pulmonary function variables ΔPplmax, RL, and Cdyn.

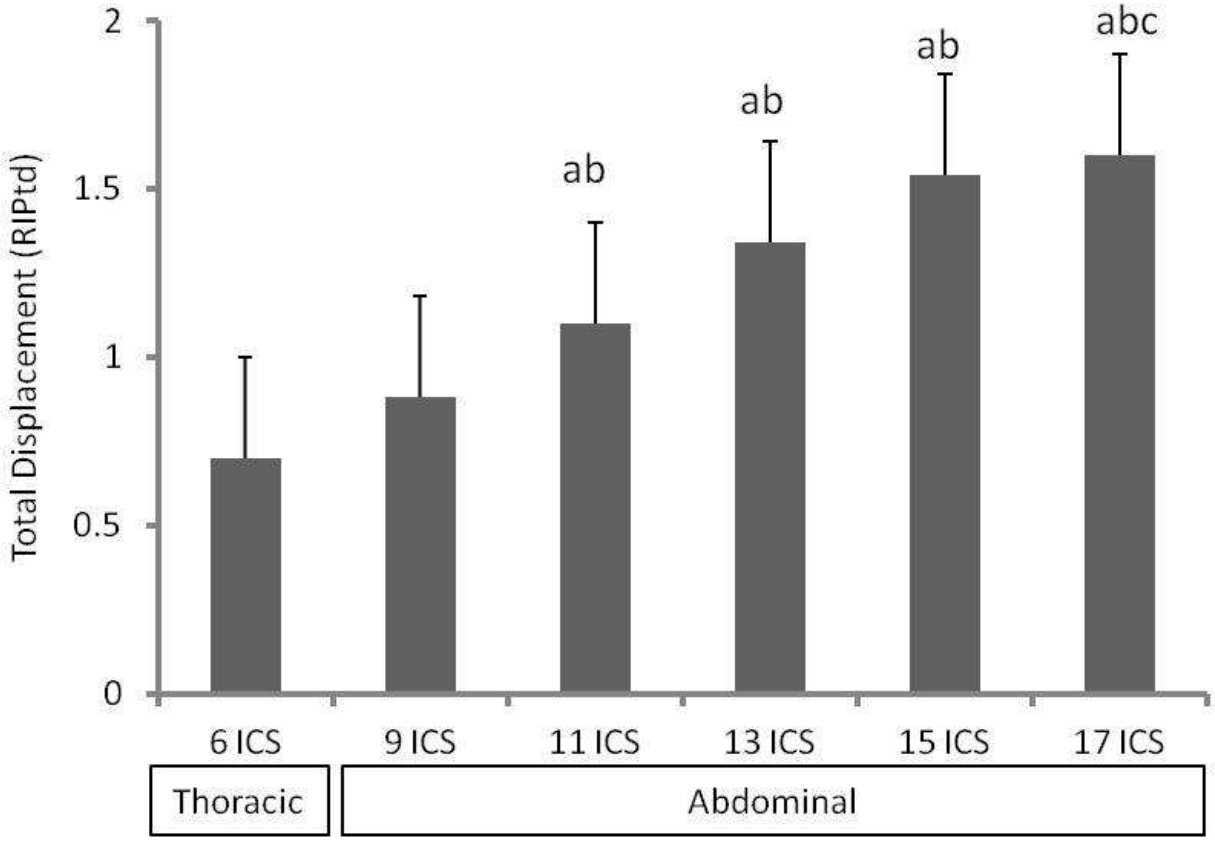



**Figure 1:** Total displacement of the thoracic band which remained at intercostal space 6 and the abdominal band that was moved among intercostals 9-17(mean ± S.E.) ICS = intercostal space; [a,b,c] significantly different from ICS 6, 9, and 11, respectively ($P<0.05$).

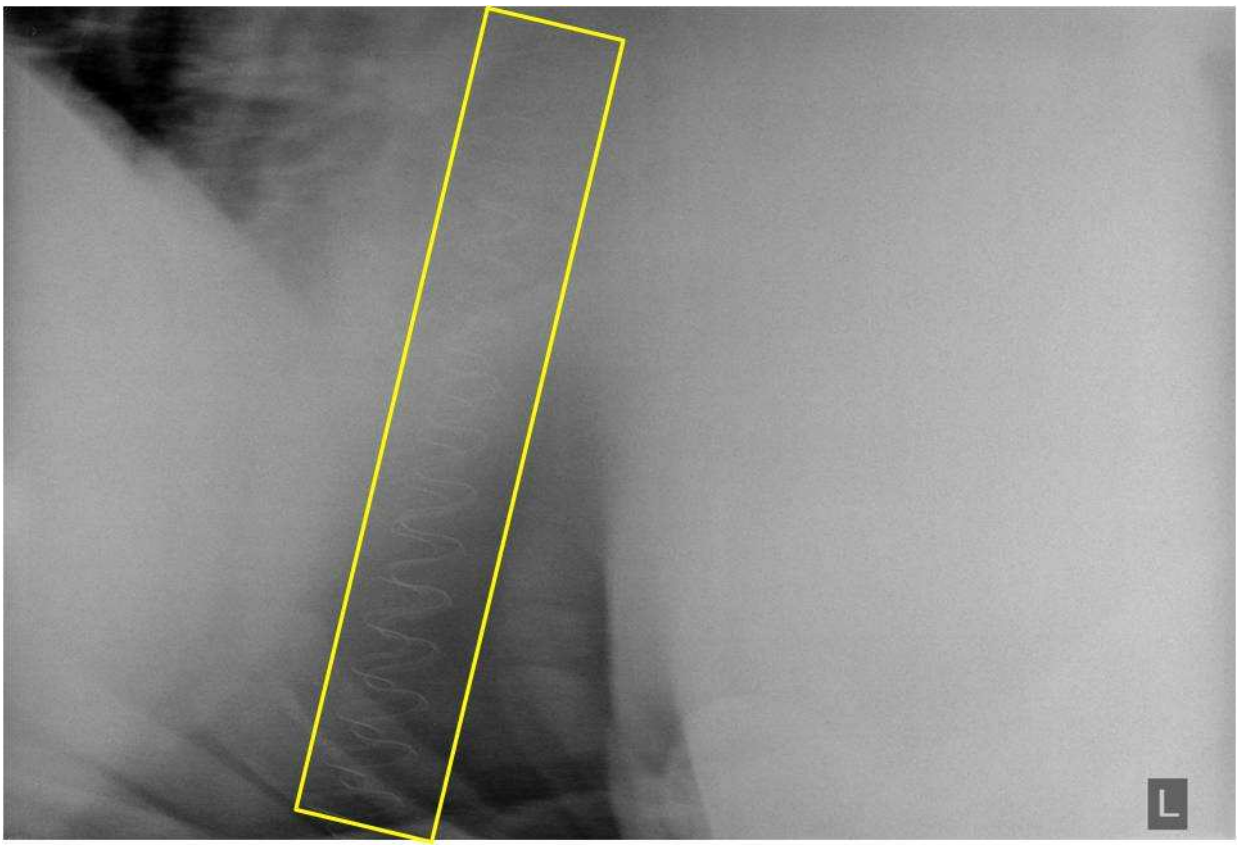
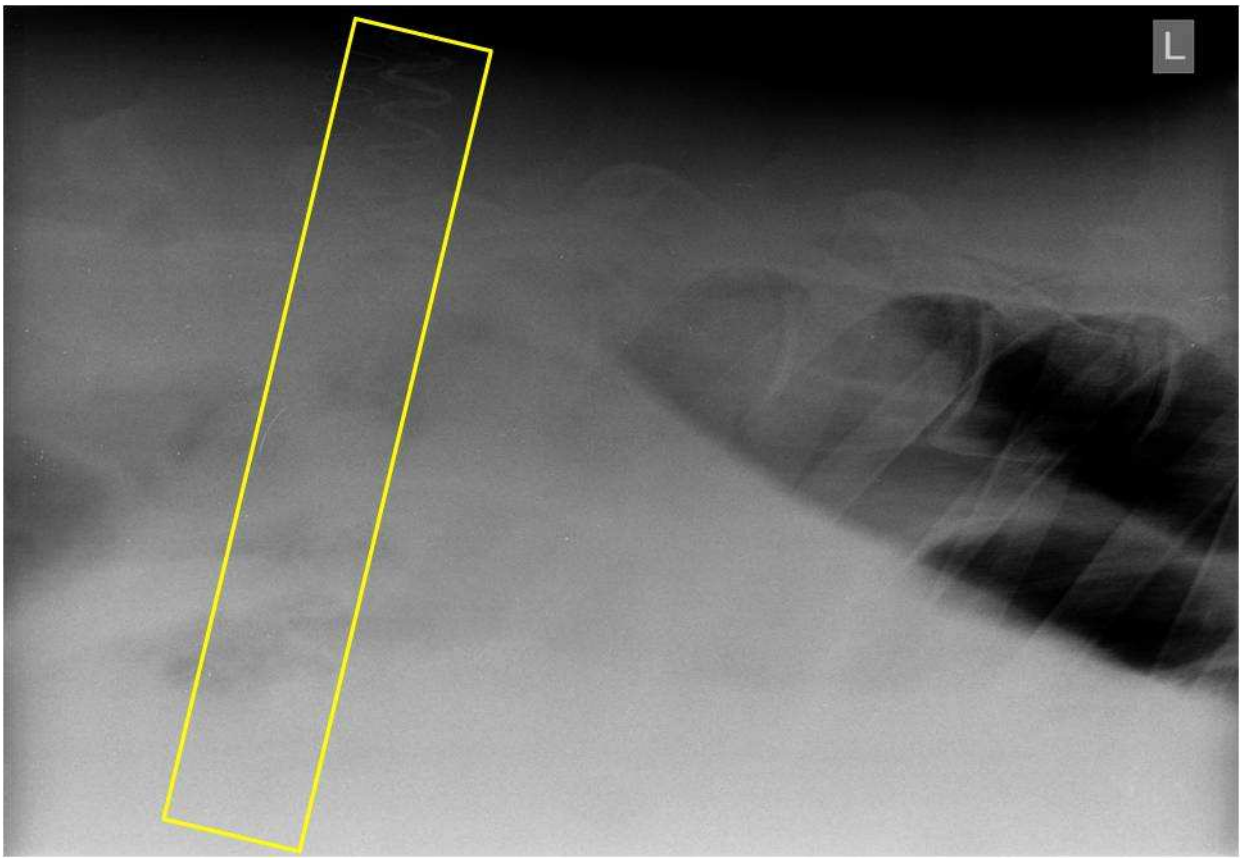



**Figure 2:** Radiograph of horse thorax and abdomen showing RIP bands at ICS 6 and ICS 17. The ICS6 and ICS17 bands overlie the thorax and abdomen, respectively. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of the thesis.

**Figure 2 (cont'd)**



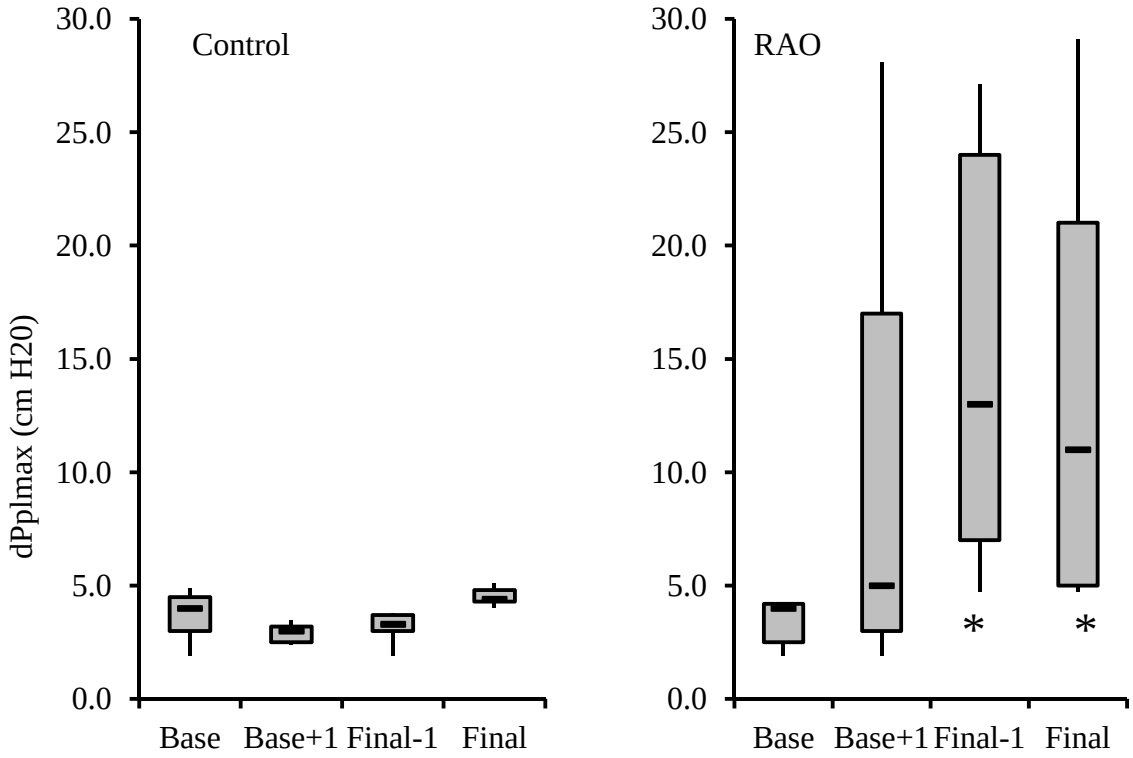



**Figure 3:** Results of pulmonary function tests from control and RAO-affected horses. Data (box and whisker plots) are shown for four days: baseline, base +1 day, final -1 day, and final day. Maximal change in pleural pressure (ΔPplmax, top), pulmonary resistance (RL, middle) and dynamic compliance (Cdyn, bottom) are shown. * indicates significantly different from control animals on the same day ($P < 0.05$).

**Figure 3 (cont'd)**

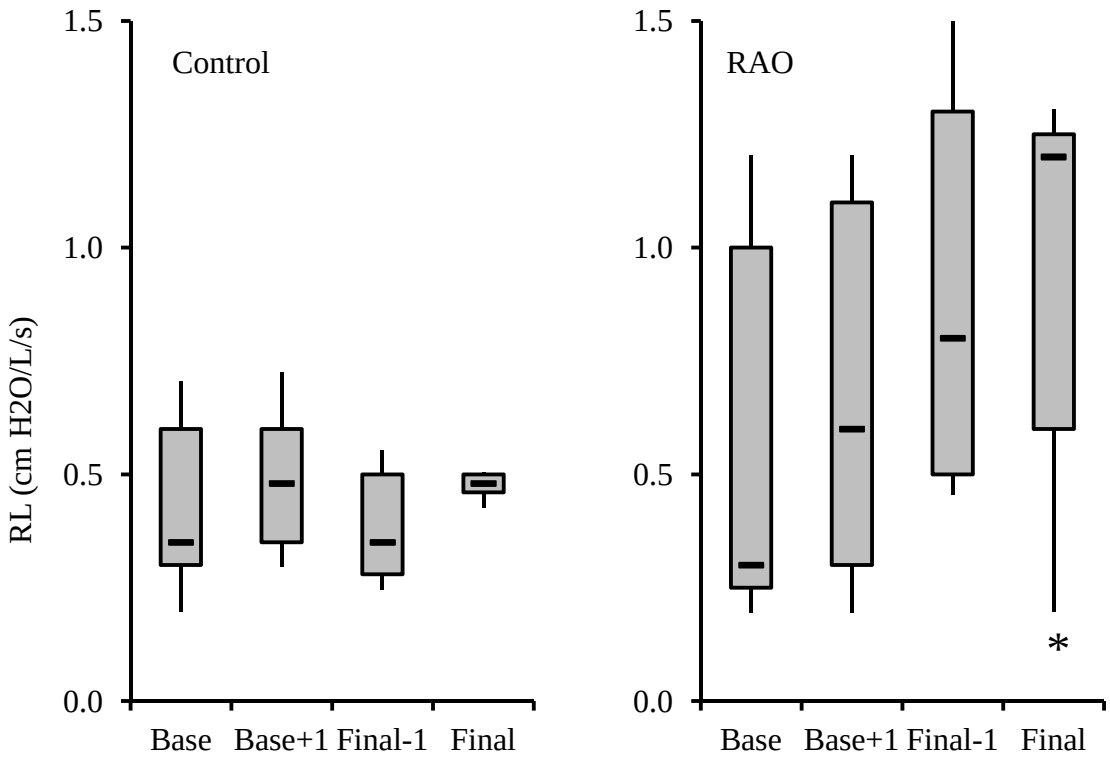
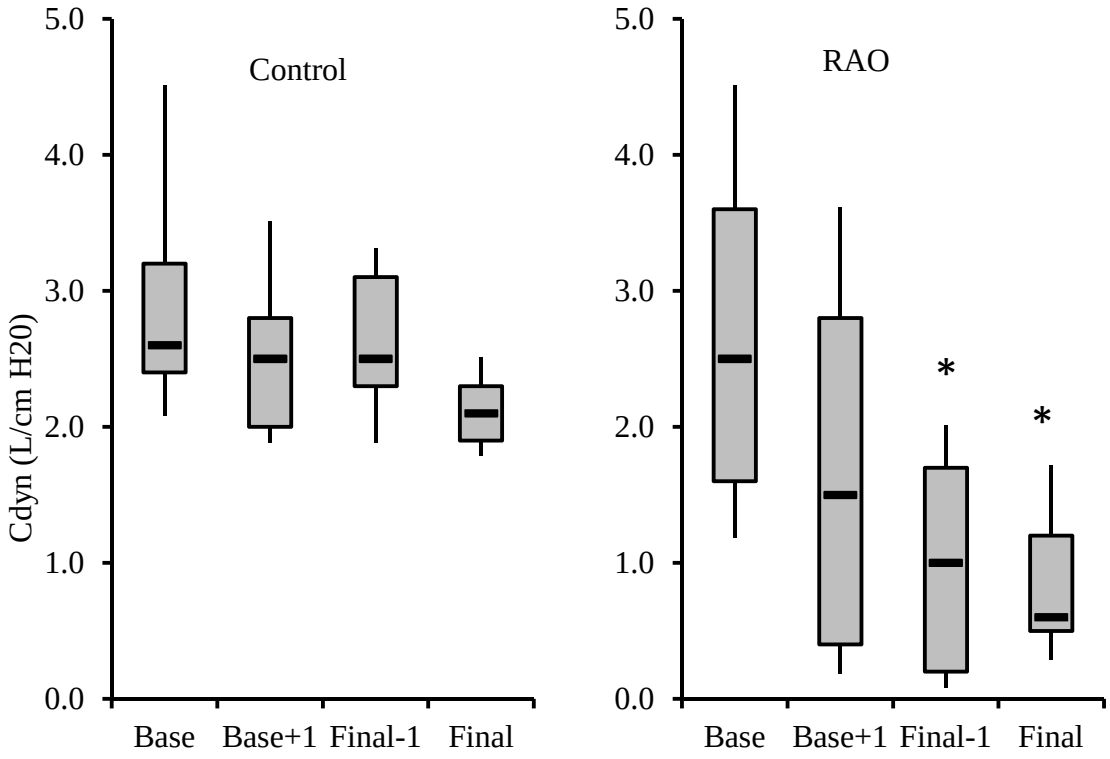


**Figure 3 (cont'd)**



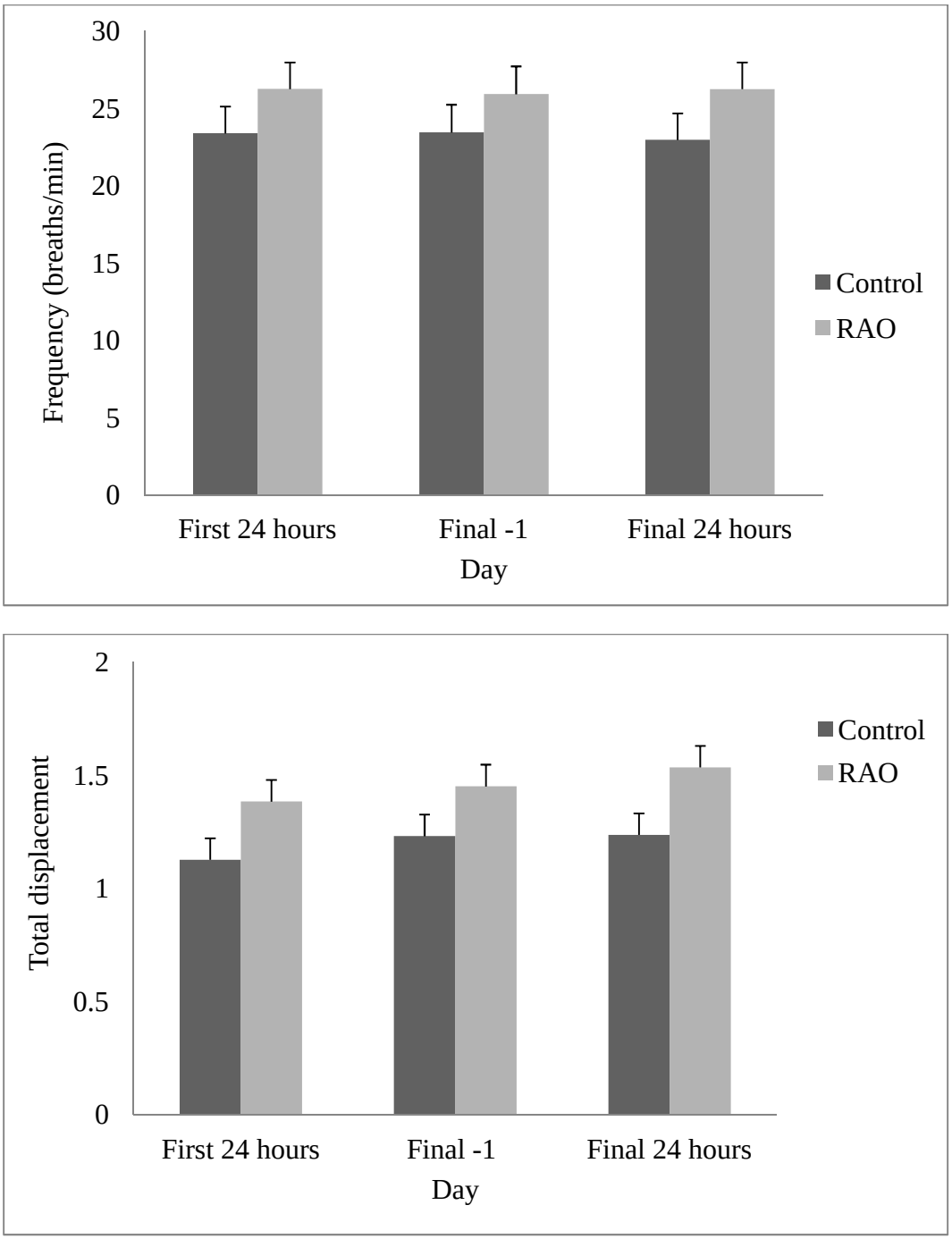



**Figure 4:** Respiratory inductance plethysmography output from control (dark bars) and RAO-affected (gray bars) horses. Data (mean ± S.E.) are shown for respiratory frequency (top) and total respiratory displacement (bottom) at three 24-hour periods – day 1, final -1 day and final day. There were no significant differences between groups or over time.

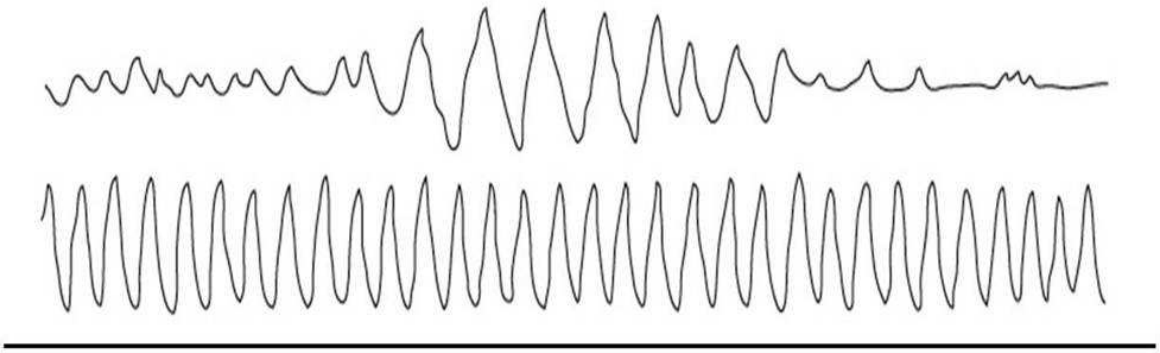


**Figure 5:** Examples of respiratory inductance plethysmography output from a control horse (top) and an RAO-affected horse with airway obstruction (bottom). These traces are of one minute recordings of the total displacement (relative Liters).

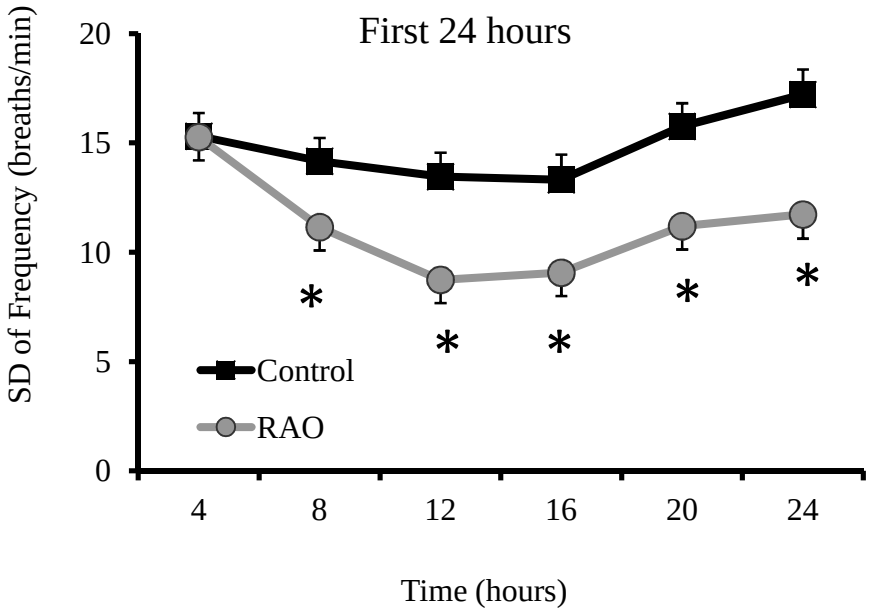
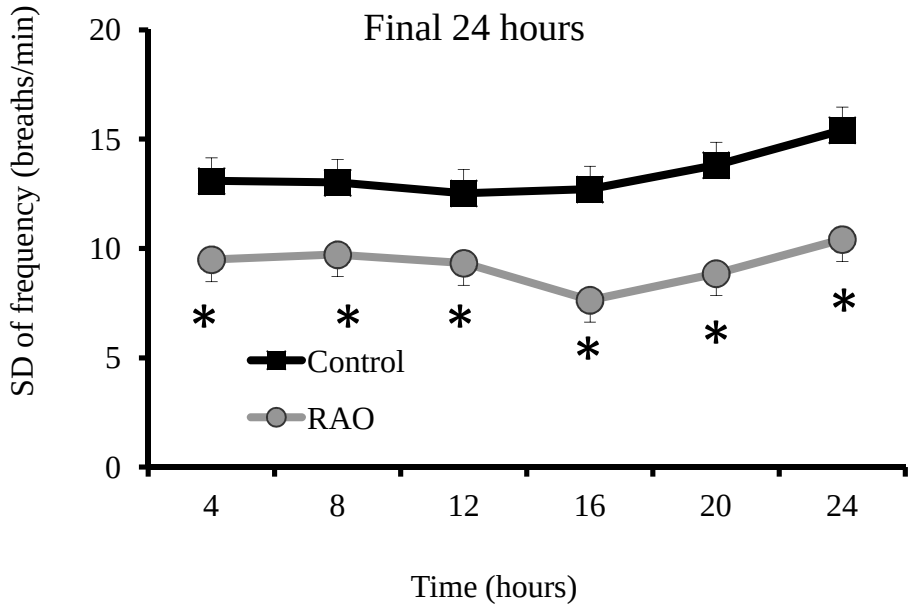



**Figure 6:** Respiratory inductance plethysmography output from control (diamonds) and RAO-affected (squares) horses. Data (mean ± S.E.) are shown for standard deviation (SD) of respiratory frequency and SD of total displacement at 4-hour intervals throughout the first and final 24-hour periods of stabling. * indicates significantly different from control animals at the same period of day ($P < 0.05$).

**Figure 6 (cont'd)**



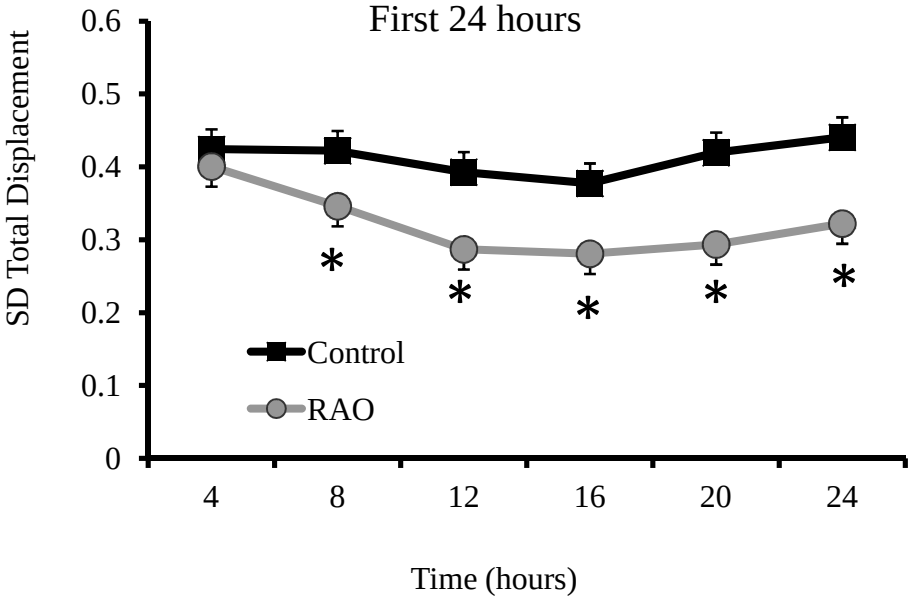
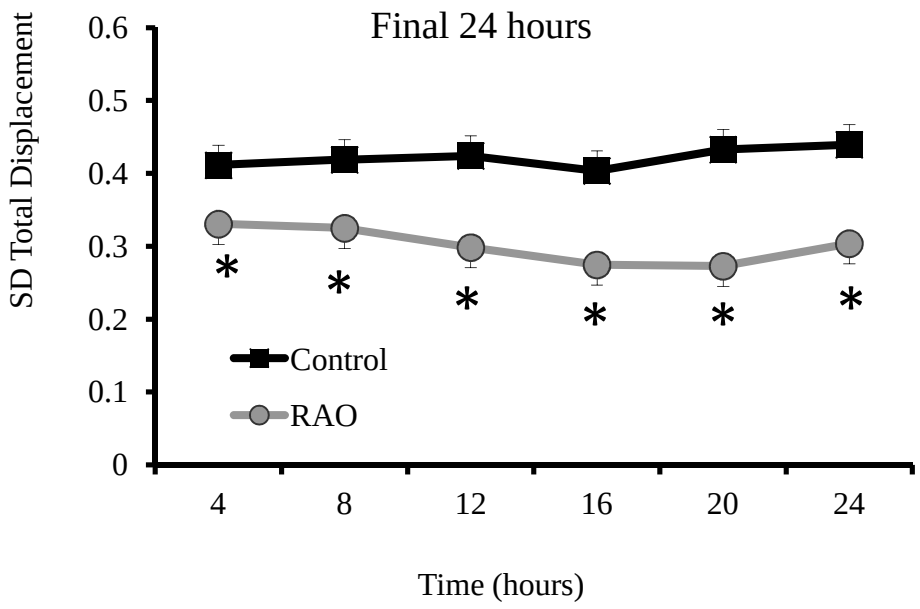



**Figure 6 (cont'd)**



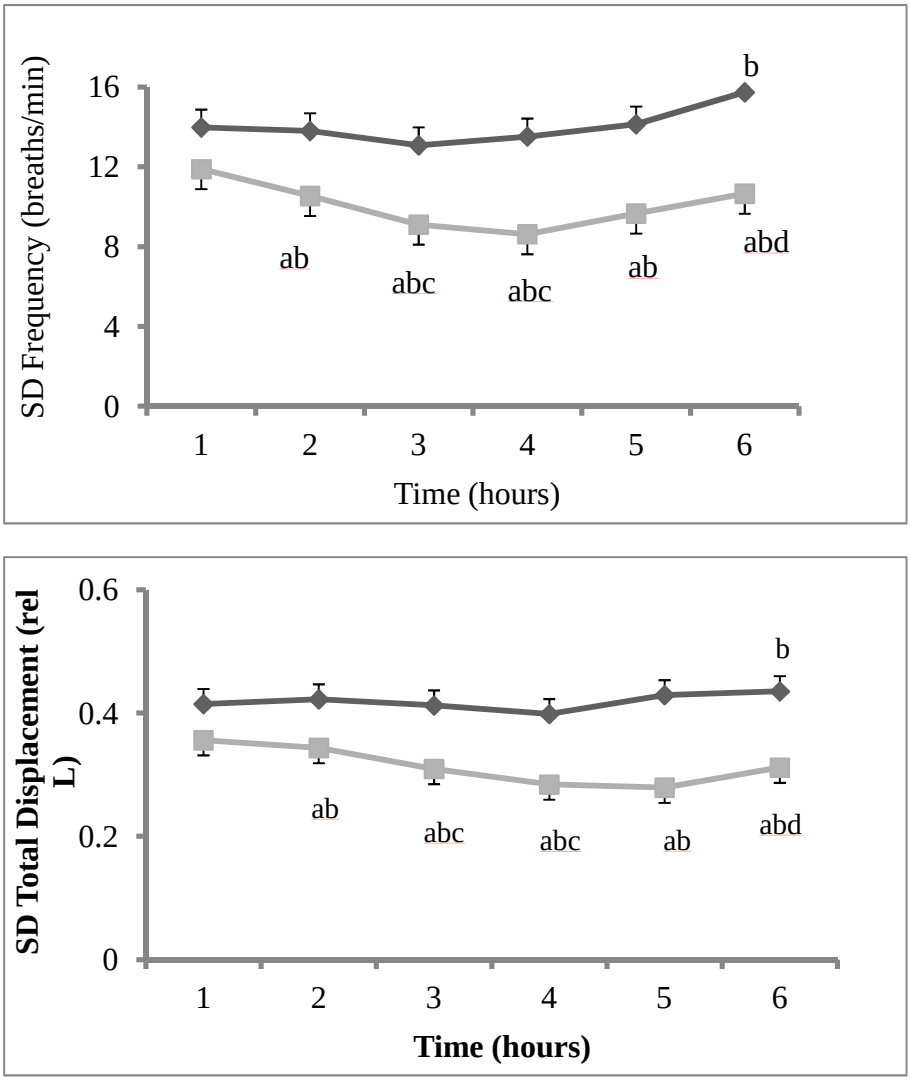



**Figure 7:** Circadian rhythm of the standard deviation (SD) of respiratory frequency (top) and total respiratory displacement (bottom). Data (mean ± S.E.) are shown at 4-hour intervals throughout the day beginning at 9 am. [a] significantly different from Period 1 in RAO-affected horses (square line) ($P<0.05$). [b] significantly different between control (diamond line) and RAO-affected horses ($P<0.05$). [c] significantly different between periods 2 and 6 ($P<0.05$).

**FOOTNOTES**

## FOOTNOTES

a. emka Technologies, Falls Church, Virginia

b. Aeromask, Trudell Medical International, London, Ontario, Canada

c. SAS, version 9.1, SAS Institute Inc, Cary, North Carolina

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