Use of the impulse oscillometry system for testing pulmonary function during methacholine bronchoprovocation in horses

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Objective—To compare sensitivity of the impulse oscillometry system (IOS) with that of the conventional reference technique (CRT; ie, esophageal balloon method) for pulmonary function testing in horses.

Animals—10 horses (4 healthy; 6 with recurrent airway obstruction [heaves] in remission).

Procedure—Healthy horses (group-A horses) and heaves-affected horses (group-B horses) were housed in a controlled environment. At each step of a methacholine bronchoprovocation test, threshold concentration (TC_{2SD}; results in a 2-fold increase in SD of a value) and sensitivity index (SI) were determined for respiratory tract system resistance (R_{rs}) and respiratory tract system reactance (X_{rs}) at 5 to 20 Hz by use of IOS and for total pulmonary resistance (R_L) and dynamic lung compliance (C_{dyn}), by use of CRT.

Results—Bronchoconstriction resulted in an increase in R_{rs} at 5 Hz (R_{5Hz}) and a decrease in X_{rs} at all frequencies. Most sensitive parameters were X_{rs} at 5 Hz (X_{5Hz}), R_{5Hz}, and R_{5Hz}:R_{10Hz} ratio; R_L and the provocation concentration of methacholine resulting in a 35% decrease in dynamic compliance ($PC_{35}C_{dyn}$) were significantly less sensitive than these IOS parameters. The TC_{2SD} for X_{rs} at 5 and 10 Hz was significantly lower in group-B horses, compared with group-A horses. The lowest TC_{2SD} was obtained for X_{5Hz} in group-B horses and R_{5Hz} in group-A horses.

Conclusions and Clinical Relevance—In contrast to CRT parameters, IOS parameters were significantly more sensitive for testing pulmonary function. The IOS provides a practical and noninvasive pulmonary function test that may be useful in assessing subclinical changes in horses. (*Am J Vet Res* 2003;64:1414–1420)

The current method that is considered the gold standard by which to evaluate respiratory mechanical function in horses is the esophageal balloon technique, a method based on simultaneous measurement of respiratory flow and pleural pressure variations during spontaneous breathing.[1] This **conventional reference technique (CRT)** has been widely used in research[2-6]; however, its application in clinical practice has been limited by its unwieldy technical requirements, as well as by the low sensitivity of measurements in instances of subclinical or mild respiratory tract diseases.[7] A need exists for sensitive lung function tests in horses to diagnose early and subclinical stages of respiratory tract diseases, as in remission of recurrent airway obstruction (ie, heaves), where affected horses do not manifest clinical symptoms. Such tests would be useful to evaluate and monitor treatment as well as in the management of such affected horses. In addition, the prevalence of lower respiratory tract diseases may be underestimated in sport horses, although their impact on performance seems to be substantial.[8,9] Investigation of lung function would thus be particularly important in these horses where pulmonary function has been shown to represent the limiting factor in performance.[10]

The **impulse oscillometry system (IOS)** is an alternative, noninvasive, forced oscillation method that has been adapted for the evaluation of pulmonary function in horses.[11] The authors have found the IOS to be a quick and easily calibrated pulmonary function test that could be readily used for routine respiratory investigation in noncooperative horses. Because the IOS generates an original, brief, pulse-shaped signal with a high-frequency content and determines respiratory impedance in a range of frequencies rather than at the unique frequency of breathing, it represents a potentially more informative test than the CRT. In humans and calves, the IOS has been shown to be significantly more sensitive than other methods for the determination of modified airway function during bronchochallenge tests.[12-14]

The purpose of the study reported here was to compare the sensitivity of the IOS against that of the CRT for performing pulmonary function testing in horses. By use of heaves-affected horses in clinical remission, we aimed at assessing the sensitivity of the IOS for the detection of subclinical respiratory dysfunction. Hence, the sensitivity and relevance of IOS measurements were compared with those obtained with the CRT during a methacholine bronchochallenge test.

Materials and Methods

Animals—Four healthy horses that were 4.5 ± 2.7 years old (mean ± SD) and weighed 482 ± 34 kg (group-A horses) and 6 heaves-affected horses that were 9.6 ± 2.3 years old and weighed 524 ± 21 kg (group-B horses) were selected for this study. Healthy horses were chosen on the basis of their history, findings on clinical examination, results of tracheobronchial and bronchoalveolar lavage fluid cytologic examinations, and results of pulmonary function tests, including arterial blood gas measurements and findings by use of the

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CRT. Preliminary studies, which included all aforementioned examinations, revealed that group-A horses were not affected by exposure to moldy hay. Heaves-affected horses were selected on the basis of their allergen response to moldy hay and the reversibility of airway obstruction by IV injection of atropine sulfate (0.04 mg/kg). Group-B horses spent 2 months on pasture to recover clinical remission. Horses of both groups were then housed indoors for 6 weeks in a controlled environment, with dust-free wood shavings as bedding and a diet of grass silage and pellets.

Prior to the protocol, the clinical status of horses with heaves was checked by clinical and respiratory endoscopic examinations, tracheobronchial and bronchoalveolar lavage fluid cytologic examination, arterial blood gas analysis, and preliminary findings by use of the CRT. All results had to be within reference range for horses to participate in the protocol.[a] The study was approved by the Animal Ethics Committee of the University of Liège, Belgium.

Impulse oscillometry system—The IOS device has been adapted to horses.[b] Briefly, an airtight facemask was placed on the horse's head in such a way as to minimize dead space and avoid compression of the nostrils. A loudspeaker produced multifrequent pressure impulses that were sent to the horse's respiratory system via a flexible tube connected to the airtight facemask. The measuring head of the IOS was placed between the flexible tube and facemask. A terminating mesh resistor (10 kPa/L/s) maintained system pressure within the measuring head while allowing the horse to breath normally. The measuring head of the IOS contained a heated pneumotachograph[c] connected to a differential pressure transducer to measure resulting airflow, and pressure signals were obtained with an identical transducer. Both transducers were phase matched up to 50 Hz and had a linear response. Flow and volume were low-pass filtered and digitized on-line by use of a computer equipped with integrated data-acquisition and analysis software.[d] Pressure and flow signals measured in the time domain were processed by Fast Fourier Transformation to obtain **respiratory system resistance (R_{rs})** and **respiratory system reactance (X_{rs})** in a range of frequencies from 5 to 20 Hz. The analysis was made on blocks of 32 points/impulse (ie, 160-millisecond sampling time) with a pulse rate of 3 pulses/s. Total duration of a single test was 30 seconds, and resulting data were averaged.

Prior to each experiment, the system was calibrated. Flow-volume calibration was performed by use of a 2-L calibration pump.[e] Pressure and impedance calibrations were then performed by use of a mesh screen reference impedance (resistance, 0.1 kPa/L/s; reactance, 0 kPa/L/s).

Conventional reference technique—Use of the CRT necessitated pleural pressure and respiratory airflow measurements for calculation of respiratory mechanical parameters. Intrapleural pressure was measured by means of an esophageal balloon catheter made from a condom sealed over the end of a polyethylene catheter (4-mm inner diameter, 6-mm outer diameter, and 220-cm length)[f] positioned with its tip in the midthoracic portion of the esophagus and connected to a pressure transducer.[g] A facemask was placed over the horse's nostrils and mouth; care was taken to minimize dead space and avoid nasal compression. A pneumotachograph[h] mounted on the facemask was coupled by 2 catheters[i] (4-mm inner diameter, 6-mm outer diameter, and 220-cm length) to a differential pressure transducer.[j] Respiratory airflow and esophageal pressure were simultaneously measured, and respiratory rate, tidal volume, **total pulmonary resistance (RL)**, and **dynamic lung compliance (Cdyn)** were calculated by a computer provided with lung function software.[k] Volume and pressure calibrations were performed with a 2-L volume pumpl and water manometer, respectively. The following baseline values were considered as reference range values for healthy horses and horses with heaves in clinical remission: RL < 0.08 kPa/L/s and Cdyn > 12 L/kPa.

Methacholine challenge—For each horse, 2 methacholine challenges were performed on successive days at the same time of day each time. Respiratory response to methacholine administered by nebulization was evaluated 1 day by use of the IOS and 1 day by use of the CRT. Order of the tests was randomly determined. During all tests, horses were restrained in stocks without chemical sedation.

Baseline pulmonary function tests were performed before each aerosol challenge. The sequence of challenges was sterile isotonic saline (0.9% NaCl) solution, followed by methacholine chloride[m] in saline solution at successive increasing concentrations (0.01 mg/mL, 0.1 mg/mL, 1 mg/mL, 2 mg/mL, and 3 mg/mL). The methacholine nebulization was stopped when the horse had signs of airway obstruction or obvious discomfort or when the highest dose of methacholine was reached. Aerosols were generated by use of an ultrasonic nebulizer.[n] Aerosolized saline solution or drugs were administered for a 1-minute period (ie, the time necessary for aerosolization of 10 mL of saline solution).[15] Pulmonary function test measurements were started 30 seconds after the end of nebulization and recorded for 3 minutes. The total time interval between each nebulization was exactly 5 minutes.

Statistical analysis—To avoid variations in respiration influencing the outcome of pulmonary function tests, breathing strategy, defined by the respiratory rate and tidal volume, was checked at baseline immediately before the methacholine challenge was performed. Results during both days were compared by use of a nonparametric Wilcoxon signed-rank test.

The following IOS parameters were used: **R_{rs} at 5, 10, 15, and 20 Hz** (**R_{5Hz}**, **R_{10Hz}**, **R_{15Hz}**, and **R_{20Hz}**, respectively) and **X_{rs} at 5, 10, 15, and 20 Hz** (**X_{5Hz}**, **X_{10Hz}**, **X_{15Hz}**, and **X_{20Hz}**, respectively). The behavior of R_{rs} and X_{rs} according to frequency (ie, frequency dependence) was also investigated, and the R_{5Hz}:R_{10Hz} ratio was determined when appropriate. For each horse, the highest rank was assigned to the **maximum dose (D_{max})** of methacholine reached, which was used to rank by number the methacholine **doses prior to D_{max}** (ie, **D_{max-1}**, **D_{max-2}**, **D_{max-3}**). This transformation improved the possibility of comparing pulmonary function test results among horses despite their varied sensitivities to methacholine challenge.

The IOS and CRT parameters were compared between group-A and group-B horses at baseline and at D_{max} by use of the nonparametric Mann-Whitney test. Baseline and D_{max} values within each group and for each pulmonary function test were compared by use of a nonparametric Wilcoxon signed-rank test. Differences in D_{max} for each group were also compared by use of a nonparametric Mann-Whitney test. For all tests, significance was set at a value of $P < 0.05$.

To compare the performance of both techniques during methacholine challenge, a **sensitivity index (SI)** was determined for each parameter, which took into account the difference between the test value and after-saline solution value and also the variability of each technique. The SI was calculated as follows:

$$SI = \frac{\text{test value - after saline value}}{SD_{ws}}$$

where SD_{ws} stands for the within-subject SD. The within-subject SD was calculated from 3 determinations of the difference in lung function measurements before and after saline solution nebulization divided by the square root of 2.

Table 1—Comparison between mean (± SD) impulse oscillometry system (IOS) and conventional reference technique (CRT) measurements of healthy adult horses (group-A horses; n = 4) and horses with clinical remission of heaves (group-B horses; 6)

		IOS					CRT	
Measurements	Horses	R_{5Hz} (kPa/L/s)	R_{10Hz} (kPa/L/s)	R_{5Hz}:R_{10Hz}	X_{5Hz} (kPa/L/s)	X_{10Hz} (kPa/L/s)	R_L (kPa/L/s)	C_{dyn} (kPa/L)
Baseline	Group A	0.076 ± 0.016	0.087 ± 0.014	0.886 ± 0.041	0.024 ± 0.004	0.007 ± 0.013	0.078 ± 0.024	16.02 ± 2.49
	Group B	0.073 ± 0.010	0.094 ± 0.013	0.781 ± 0.053	0.026 ± 0.004	0.017 ± 0.006	0.065 ± 0.011	18.04 ± 4.23
D_{max}	Group A	0.114 ± 0.012*	0.101 ± 0.017	1.143 ± 0.161*	–0.010 ± 0.007*	–0.013 ± 0.003*	0.160 ± 0.026*	6.36 ± 1.08*
	Group B	0.101 ± 0.021*	0.097 ± 0.016	1.059 ± 0.087*	–0.022 ± 0.026*	–0.015 ± 0.020*	0.160 ± 0.047*	9.28 ± 2.96*

*Significant ($P < 0.05$) difference between D_{max} and baseline values.

D_{max} = Maximum dose of methacholine. R_{5Hz} = Respiratory system resistance at 5 Hz. R_{10Hz} = Respiratory system resistance at 10 Hz. R_{5Hz}:R_{10Hz} = Ratio of R_{5Hz} to R_{10Hz} (used to express frequency dependence of respiratory system resistance in the low-frequency range). X_{5Hz} = Respiratory system reactance at 5 Hz. X_{10Hz} = Respiratory system resistance at 10 Hz. R_L = Total pulmonary resistance. C_{dyn} = Dynamic lung compliance.

The SI expresses the absolute changes in lung function measurements during challenge in multiples of the baseline reproducibility of the given technique, thus enabling direct comparison of measurements by both techniques despite differences in baseline reproducibility.[16]

The **threshold concentration that results in a 2-fold increase in the SD** (TC_{2SD}) of lung function measurements was also estimated. Linear interpolation was performed between the values surrounding the TC_{2SD} value for each horse and pulmonary function test. To allow comparison with previously published work,[2,17] the **provocation concentration of methacholine resulting in a 35% decrease in dynamic compliance** ($PC_{35}C_{dyn}$) was also determined.

The sensitivity of each parameter for each technique was assessed from the SI and TC_{2SD}. The higher the SI and the lower the TC_{2SD}, the higher the sensitivity of the considered parameter for detection of methacholine-induced bronchoconstriction.

Differences in TC_{2SD} across group-A and group-B horses and among parameters were assessed with a 2-way ANOVA with interaction. Correlation between IOS and CRT parameters was estimated for all steps of the challenge. Null hypotheses of no difference in TC_{2SD} and SI and no correlation between TC_{2SD} and SI were rejected when P values were < 0.05.

Results

Although they were not sedated, all group-A and group-B horses complied well with the test procedures and methacholine-challenge test. Breathing strategy did not differ significantly on the days the tests were performed (data not shown). The D_{max} attained for each horse on consecutive days was identical. However D_{max} values were significantly lower in group-B horses (mean D_{max}, 1.33 mg/mL), compared with group-A horses (mean D_{max}, 2.75 mg/mL). No significant differences in parameters determined by use of IOS or CRT were found between group-A and group-B horses at baseline or D_{max} (**Table 1**).

IOS parameters—Frequency dependence of R_{rs} and X_{rs} in the lower-frequency range (5 to 10 Hz) was progressively modified during methacholine inhalation (**Fig 1**), as R_{rs} became negatively dependent on frequency and X_{rs} became positively dependent on frequency. The IOS parameters that had the highest degree of change during the course of the methacholine challenge were R_{rs} and X_{rs} at 5 and 10 Hz. Because of this finding, only R_{5Hz}, R_{10Hz}, X_{5Hz}, and X_{10Hz} were retained for further analysis.

Comparison between IOS and CRT parameters— No significant differences were found in the SI between

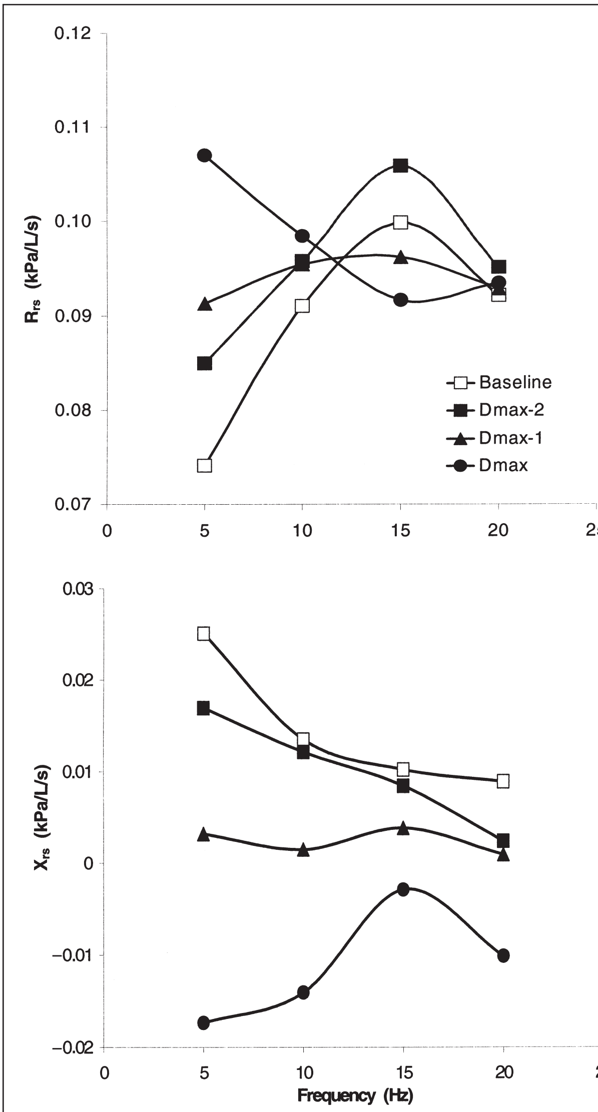

Figure 1—Mean values of respiratory system resistance (R_{rs}; top panel) and reactance (X_{rs}; bottom panel) according to frequency as measured by use of the impulse oscillometry system at baseline and during inhalation of increasing doses of methacholine. The highest rank was assigned to the maximum dose (D_{max}) of methacholine, and decreasing ranks were assigned to doses of methacholine prior to D_{max} (ie, $D_{max–1}$ and $D_{max–2}$). Mean values were determined from 10 horses (4 healthy adult horses and 6 horses with clinical remission of heaves).

Table 2—Comparison of mean (± SD) threshold concentration of methacholine that results in a 2-fold increase in the SD (TC_{2SD}) of IOS and CRT indices of healthy adult horses (group-A horses; n = 4) and horses with clinical remission of heaves (group-B horses; 6)

Horses	TC_{2SD} (mg/mL)							
	R_{5Hz}	R_{10Hz}	R_{5Hz}:R_{10Hz}	X_{5Hz}	X_{10Hz}	R_L	C_{dyn}	$PC_{35}C_{dyn}$
Group A	0.53 ± 0.34[a]	1.16 ± 0.45[a]	0.35 ± 0.12[a]	0.84 ± 0.26[b,c]	1.65 ± 0.46	2.33 ± 0.44	1.83 ± 0.99	1.95 ± 0.34
Group B	0.35 ± 0.16	1.55 ± 0.33[a]	0.34 ± 0.17	0.12 ± 0.09*[b,c]	0.41 ± 0.21*	0.65 ± 0.27*	0.74 ± 0.31	1.01 ± 0.42*

*Significant ($P < 0.05$) difference between group-A and group-B horses.
[a,b,c]Two-fold increase in the SD values that are significantly ($P < 0.05$) different from those of R_L, C_{dyn}, and $PC_{35}C_{dyn}$, respectively, for each group of horses.
$PC_{35}C_{dyn}$ = Provocation concentration of methacholine resulting in a 35% decrease in dynamic compliance.
See Table 1 for remainder of key.

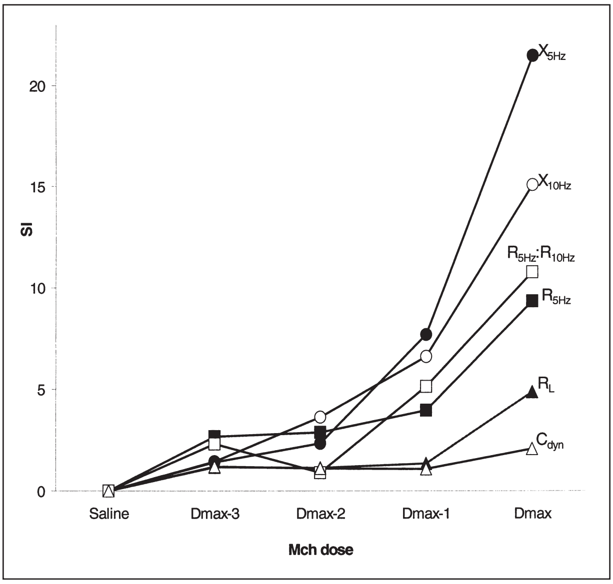

Figure 2—Dose-response curve of the change in pulmonary function parameters, expressed as sensitivity index (SI), versus increasing doses of inhaled methacholine following inhalation challenge with saline solution. X_{5Hz} = Respiratory system reactance at 5 Hz. X_{10Hz} = Respiratory system reactance at 10 Hz. R_{5Hz}:R_{10Hz} = Ratio of R_{5Hz} to R_{10Hz}. R_{5Hz} = Respiratory system resistance at 5 Hz. R_L = Total pulmonary resistance. C_{dyn} = Dynamic lung compliance. *See* Figure 1 for remainder of key.

group-A and group-B horses, and final results were determined for all 10 horses (**Fig 2**). A high within-subject SD by use of CRT contributed to a higher SI for R_L and C_{dyn}, compared with SI obtained for the same parameters determined by use of IOS. The sensitivity of the measured parameters ranked as follows: $X_{5Hz} > X_{10Hz} > R_{5Hz}:R_{10Hz} > R_{5Hz} > R_{10Hz} > R_L > C_{dyn}$. The SI for X_{5Hz} was significantly higher than the SI for R_{5Hz}, R_{10Hz}, R_L, and C_{dyn}, but was not significantly different from the SI for X_{10Hz} or the SI for the R_{5Hz}:R_{10Hz} ratio.

The TC_{2SD} for both groups of horses was determined by use of the IOS and CRT (**Table 2**). With the exception of R_{10Hz}, all the TC_{2SD} values obtained for IOS parameters within each group of horses were lower than any of the TC_{2SD} values obtained for CRT parameters. Challenge dose-response curves for R_{5Hz}, R_{5Hz}:R_{10Hz} ratio, and X_{5Hz} revealed the lowest threshold concentration of methacholine for both horse groups.

Comparison between healthy and heaves-affected horses in remission—In group-B horses, theTC_{2SD} for X_{5Hz}, $X_{10Hz,}$ and R_L and the $PC_{35}C_{dyn}$ were significantly lower than in group-A horses. In group-B horses, although the $PC_{35}C_{dyn}$ was not significantly different from the TC_{2SD} for R_L and TC_{2SD} for C_{dyn}, it corresponded to the highest concentration and was thus considered the least sensitive CRT parameter for group-B horses.

Significant correlations were found between X_{5Hz} and C_{dyn}, X_{10Hz} and C_{dyn}, and R_{5Hz} and R_L; however, the *r* values were low (*r* = 0.29, 0.34, and 0.58, respectively). However, correlation evaluations performed with mean IOS and CRT parameters at baseline and with increasing doses of methacholine had significantly higher *r* values. A positive correlation was found between R_{5Hz} and R_L (*r* = 0.76) and between X_{5Hz} and C_{dyn} (*r* = 0.93), whereas X_{5Hz} and R_L (*r* = 0.75) were negatively correlated. Values of X_{rs} and R_{rs} measured beyond 5 Hz were not significantly correlated with CRT parameters.

Discussion

To determine whether the IOS could be used to effectively detect various degrees of obstruction of the lower airways in horses, we chose to induce lower airway constriction by nebulizing increasing doses of methacholine. Use of methacholine inhalation to induce nonspecific airway constriction in horses has been widely documented.[2,18,19] Methacholine provokes smooth-muscle contraction in the airway by direct interaction with muscarinic receptors and is a potent constrictor of central and peripheral airways and to a lesser extent of lung parenchyma in horses.[20] Because the maximal effect of inhaled methacholine on respiratory mechanics is short-lived and its onset varies from 1 individual to another,[21] simultaneous IOS and CRT testing was impossible to perform, and we chose to evaluate each method on 2 separate consecutive days. As variability in minute ventilation may affect results of methacholine-challenge tests in certain horses over several days,[21,22] respiratory frequency and tidal volume were evaluated prior to challenge and compared for each horse. The absence of significant changes in respiratory minute pattern and the achievement of identical D_{max} values for each horse on both days of challenge support the fact that intraindividual respiratory data were repeatable and comparable during our study. As the time between each nebulization step was relatively short, a cumulative effect of methacholine was obtained.[21,23]

The IOS parameters to have the most significant changes with methacholine-induced airway obstruction were X_{rs} and R_{rs} in the lower-frequency range of 5 and 10 Hz. As a result, frequency-dependent behavior of both parameters was also significantly modified by methacholine challenge. The negative frequency dependence of R_{rs} and the decrease in X_{rs} in the lower-frequency range are characteristic of lower airway obstruction, which has been shown in horses with clinical exacerbation of heaves.[11,24] The X_{rs} values in the lower-frequency range are mainly dependent on dynamic and static compliance properties of the respiratory system. Consequently, these X_{rs} values are influenced by peripheral resistance and heterogeneity of time constants[25] and by the elastic properties of lung tissue and chest wall.[26] In the lower-frequency range, R_{rs} values are mainly determined by the caliber of central and lower airways.[27] By modifying central and lower airway patency[21] and increasing ventilation heterogeneity,[28] inhalation of increasing doses of methacholine is responsible for the progressive increase of R_{5Hz} and decrease of X_{rs} in the low-frequency range, as observed in our study. Hence, IOS parameters in the low-frequency range are sensitive to methacholine-induced airway constriction.

To express frequency dependence, ratios of R_{rs} at specific frequencies have been shown to better discriminate between physiologically normal conditions and conditions of lower airway obstruction, in comparison to linear regression calculations.[24] For this reason, we chose to represent frequency dependence of R_{rs} by the R_{5Hz}:R_{10Hz} ratio. In our study, the R_{5Hz}:R_{10Hz} ratio proved to be a sensitive indicator of early methacholine-induced bronchoconstriction. Because of the greater variability of X_{rs} and its variation around 0, calculation of ratios or differences to define frequency dependence for this parameter was inappropriate.

Although previous publications relate **forced oscillation technique (FOT)** measurements in a lower-frequency spectrum for the evaluation of airway obstruction in horses,[24,29] our data confirm the relevance of investigating R_{rs} and X_{rs} at 5 Hz and greater by use of the IOS. Results of a recent study[30] comparing the IOS to a pseudorandom noise FOT in human patients revealed that there is an upward shift in the resonant frequency measured by use of the IOS, compared with that estimated by use of the FOT. A similar shift in frequencies between the FOT described by Young et al[24] and the IOS could explain the relevance of performing measurements in a higher-frequency spectrum with the latter. Values of R_{rs} and X_{rs} beyond 15 Hz were not modified by changes in lower airway function. In a previous study,[11] we have shown that values of R_{rs} and X_{rs} at 15 to 35 Hz probably reflected the mask measuring device and upper airways.

Because of differences in data acquisition and calculations, direct comparison between IOS and CRT parameters is inappropriate. Because of this, as well as the large interindividual variability in baseline measurements and lack of sensitivity of CRT parameters in several horses during the methacholine-challenge test, mediocre *r* values were obtained when correlations between IOS and CRT parameters were performed on pooled data (*r* values of 0.29 to 0.64). However, correlation between averaged IOS and CRT data for each step of the methacholine challenge was good, indicating that both techniques detected modifications in airway caliber during the methacholine inhalation challenge in a proportional manner. As signal penetration is inversely proportional to frequency, parameters in the lower frequencies reflect the total respiratory system. Consequently, R_{rs} and X_{rs} at 5 Hz were the IOS parameters most strongly correlated with R_L and C_{dyn}.

To our knowledge, our study is the first to evaluate and compare sensitivity of an FOT and the gold standard CRT in horses. All the TC_{2SD} values obtained for relevant IOS parameters were lower than any of the TC_{2SD} values obtained for the CRT parameters, indicating that the IOS detected earlier changes in airway function during the course of the methacholine challenge than did the CRT. The determination of a threshold concentration was preferred to that of a provocation concentration causing a predetermined change in lung function (expressed as percentage change), because the threshold concentration is calculated from individual baseline lung function, whereas provocation concentration is arbitrarily chosen. To facilitate comparison between parameters of both pulmonary function tests, SI, indicating the absolute increase in multiples of the baseline variability, was estimated. Taking SI and TC_{2SD} into account, we have found that the IOS is a significantly more sensitive method than the CRT for the detection of lower airway obstruction in horses because the IOS parameters revealed both earlier and smaller changes in pulmonary function, compared with the CRT parameters. In our study, the measurements of pulmonary function in the frequency domain proved to be particularly interesting, as R_{rs} and X_{rs} at 5 Hz were markedly modified in relation to values at higher frequencies, and with X_{5Hz} and R_{5Hz}, the R_{5Hz}:R_{10Hz} ratio was the most sensitive parameter to estimate methacholine-induced bronchoconstriction. In contrast, estimated CRT parameters had the least sensitivity to alterations in airway function. Although $PC_{35}C_{dyn}$ is commonly used for monitoring the response to bronchial challenge,[2,17] results of our study indicate that it is 1 of the least sensitive parameters both in healthy and heaves-affected horses.

Observation of a lower D_{max} in group-B horses, compared with group-A horses, suggests that, as previously reported,[17,31] heaves-affected horses sustain airway hyperreactivity even when housed in a controlled environment. Use of both the IOS and CRT allowed discrimination between heaves-affected horses in clinical remission and healthy horses when submitted to the bronchochallenge test. The TC_{2SD} values were generally lower in group-B horses, compared with group-A horses, the difference being significant for X_{5Hz}, X_{10Hz}, and R_L, indicating that the onset of methacholine-induced response occurred earlier in heaves-affected horses.

The IOS measurements determined in our study also seem to indicate that there are differences in the properties of the response to inhaled methacholine in heaves-affected and healthy horses. Indeed, the lowest TC_{2SD} was found for R_{5Hz} in group-A horses, whereas the TC_{2SD} for X_{5Hz} was the lowest value in group-B horses (and significantly lower than the TC_{2SD} for X_{5Hz} obtained in group-A horses), indicating that the first parameter to change with methacholine inhalation was R_{5Hz} in healthy horses and X_{5Hz} in heaves-affected horses. In a recent study[14] conducted in asymptomatic smokers, a significant decrease of X_{5Hz} measured by use of the IOS was found after a methacholine challenge, without concomitant changes in R_{rs}. The behavior of X_{5Hz} was attributed to diffuse subclinical bronchiolitis.[14] Modified local airway and tissue structures have been previously suggested to participate in the pathogenesis of hyperreactivity in diseased horses.[32] Results of a histologic study[o] reveal that epithelial remodeling could also be present in equine airways without concurrent signs of inflammation. The X_{rs} values in heaves-affected horses could have been caused by underlying alterations in airway and tissue structures. The occurrence of methacholine-induced bronchospasm on altered peripheral airway structures could accentuate the initial variation of X_{rs} in heaves-affected horses, even those in clinical remission of the disease, as observed in our study. The IOS may therefore provide a sensitive pulmonary function test to assess the repercussions of mild peripheral airway and lung tissue alterations in horses. Contrary to the CRT, the IOS may also be useful for determining the degree of dysfunction in the respiratory system in response to inhaled methacholine and to distinguish between healthy horses and heaves-affected horses in clinical remission. The application of mathematical models to the IOS data based on physical equivalences between respiratory and electrical structures could be useful to further explore this aspect of the IOS.[33,34]

The superior sensitivity of the IOS, compared with the CRT, and its potential ability to locate functional impairment could make this pulmonary function test particularly interesting for the investigation of pathogenesis and the therapeutic drug targeting of respiratory tract diseases. Moreover, because of its undeniable practical advantages, the IOS could be used for clinical bronchochallenge testing in the early detection of subclinical respiratory tract diseases in horses.

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Correction: In the article "Macroscopic changes in the distal ends of the third metacarpal and metatarsal bones of Thoroughbred racehorses with condylar fractures," which was published in the September 2003 issue of the *American Journal of Veterinary Research* (2003;64:1110–1116), the image labeled Figure 2B was incorrect. The correct images for Figure 2 are reproduced below.

Figure 2—Photographs of the distal end of the third metatarsal bone from a Thoroughbred racehorse euthanatized because of a condylar fracture in the contralateral limb (A) and of the distal end of the third metacarpal bone from a nonracehorse (B). Parasagittal linear erosions are seen in the articular cartilage of the lateral and medial condylar grooves (A; white arrows) of the bone from the racehorse, as well as a circular erosion in the articular cartilage of the lateral condyle (black arrows); parasagittal wear lines in the articular cartilage are also seen. Parasagittal wear lines are not seen in the nonracehorse (B).