The effect of aminophylline on trans-diaphragmatic pressure in isoflurane anaesthetised dogs undergoing castration

K. PAVLIDOU, I. SAVVAS, Y. MOENS, G. KAZAKOS, D. RAPTOPOULOS

To cite this article:

The effect of aminophylline on trans-diaphragmatic pressure in isoflurane anaesthetised dogs undergoing castration

Pavlidou K.[1], Savvas I.[1*], MoensY.[2], Kazakos G.[1], Raptopoulos D.[1]

[1] Unit of Anaesthesiology and Intensive Care, Companion Animal Clinic, School of Veterinary Medicine,

Faculty of Health Sciences, Aristotle University of Thessaloniki

[2] Anaesthesiology and perioperative Intensive Care, University of Veterinary Medicine, Vienna, Austria

ABSTRACT. The trans-diaphragmatic pressure (P_{di})is the main indicator of diaphragmatic contractility and the influence of many different drugs on P_{di} has already been studied. The main aim of this study was to investigate the effect of aminophylline on P_{di} in anaesthetised dogs. Eighteen, healthy, client-owned, male dogs undergoing castration were recruited in the study. All the animals were premedicated with dexmedetomidine and morphine. Anesthesia was induced with propofol to effect and maintained with isoflurane in oxygen. Animals were randomly allocated into two groups. In the animals of the aminophylline group (group A), aminophylline was administered as an intravenous bolus at 4mg/kg after induction, whereas in the animals of the control group (group C), no aminophylline was given. When the surgical plane of anesthesia was achieved, two balloon catheters, one in the stomach and one in the mid-third of the oesophagus were inserted for P_{di} measurement. The two groups differed non-significantly with regards to P_{di} (p=0.182). The results of the study could be viewed as an indication that the aminophylline might increase diaphragmatic contractility if also proven in a larger population of animals'.

Keywords: diaphragm, trans-diaphragmatic pressure, oesophageal balloon catheters, dog

ΠΕΡΙΛΗΨΗ. Η δια-διαφραγματική πίεση (P_{di}) είναι ο κύριος δείκτης της συσπαστικότητας του διαφράγματος και η επίδραση αρκετών διαφορετικών φαρμάκων σε αυτήν έχει μελετηθεί. Ο κύριος στόχος αυτής της μελέτης είναι να μελετηθεί η επίδραση της αμινοφυλλίνης στη P_{di} σε σκύλους υπό αναισθησία. Σε αυτή την μελέτη συμπεριλήφθηκαν δεκαοχτώ, υγιείς, ιδιόκτητοι, αρσενικοί σκύλοι οι οποίοι υποβλήθηκαν σε ορχεκτομή. Η προαναισθητική αγωγή σε όλα

Corresponding Author:
Ioannis Savvas, Unit of Anaesthesiology and Intensive Care, Companion Animal Clinic, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, St. Voutyra 11, GR-54627, Thessaloniki, Greece
Email:isavas@vet.auth.gr

τα ζώα ήταν η δεξμεδετομιδίνη και η μορφίνη. Η εγκατάσταση της αναισθησίας έγινε με προποφόλη και η διατήρηση με ισοφλουράνιο σε οξυγόνο. Τα ζώα κατανεμήθηκαν τυχαία σε δύο ομάδες. Στην ομάδα της αμινοφυλλίνης (ομάδα Α), η χορήγηση της αμινοφυλλίνης γινόταν ενδοφλέβια ως μια δόση στα 4 mg/kg αμέσως μετά την εγκατάσταση της αναισθησίας, ενώ στην ομάδα control (ομάδα C) δεν χορηγούνταν αμινοφυλλίνη. Όταν η χειρουργική αναισθησία επιτυγχανόταν, εισάγονταν δύο καθετήρες με μπαλόνι, ο ένας στο στόμαχο και ο άλλος στο μέσο τρίτο του οισοφάγου, για τη μέτρηση της P_{di}. Οι δύο ομάδες δε διέφεραν στατιστικά σημαντικά όσον αφορά τη P_{di} (p=0,182). Τα αποτελέσματα της μελέτης θα μπορούσαν να αποτελούν μια ένδειξη ότι η αμινοφυλλίνη ίσως αυξάνει την συσπαστικότητα του διαφράγματος εάν αποδειχθεί σε μεγαλύτερο πληθυσμό ζώων.

Λέξεις-κλειδιά: διάφραγμα, δια-διαφραγματική πίεση, οισοφαγικοί καθετήρες, σκύλος

INTRODUCTION

The diaphragm is the main inspiratory muscle and its normal function is of great importance for the anesthesiologist. Diaphragmatic insufficiency, especially diaphragmatic fatigue, has been associated with many respiratory diseases in humans and animals. Moreover, several pharmacological agents, such as anesthetic drugs (propofol, halothane, isoflurane) have been found to depress diaphragmatic contractility in experimental studies (Jagers et al.2009; Kochi et al. 1992; Nishina et al.2003; Zhang et al.2009). In veterinary clinical practice, the effect of four anesthetic drugs (isoflurane, fentanyl, propofol, ketamine) on diaphragmatic contractility in dogs has already been studied (Pavlidou et al. 2013).

Methylxanthines have several pharmacological actions of therapeutic interest. They relax smooth muscles, especially the bronchial muscles, and they stimulate the central nervous system (Jagers et al. 2009). They are also positive chronotropes and inotropes, as well as mild diuretics. In human medicine, theophylline is used to increase contractility of fatigued diaphragm in healthy patients and in patients with chronic obstructive pulmonary disease (Aubier 1985; Jagers et al. 2009; Sigrist et al. 1982). In small animal clinical practice, theophylline and aminophylline are used as bronchodilators and they have also been used experimentally to improve diaphragmatic contractility in both normal and fatigued diaphragm in dogs (Aubier et al. 1983b; Gayan-Ramirez et al. 1994; Sigrist et al.1982).

Aminophylline is a methylxanthine, which has been widely studied in human and veterinary clinical practice. In one study it has been shown that aminophylline may increase trans-diaphragmatic pressure (P_{di}) in a fatigued human diaphragm (Aubier et al. 1981), while another study has failed to support this finding (Levy et al. 1990). Moreover, theophylline seems to improve exercise capability in patients with chronic obstructive pulmonary disease (Murciano et al.1984). *In vivo* studies in anaesthetised laboratory animals and *in vitro* studies in skeletal muscle fibers support the significant positive effect of methylxanthines on diaphragmatic contractility (Aubier 1985; Jagers et al.2009; Levy et al.1990; Wanke et al.1994). It has been shown that in anesthetized dogs, inspiratory muscle contractility was improved in a dose-dependent manner after the administration of aminophylline in a paralyzed diaphragm (Sigrist et al.1982).

Trans-diaphragmatic pressure (P_{di}) is a very good indicator of diaphragmatic contractility in clinical practice (Laporta and Grassino 1985). The measurement of P_{di} during maximum inspiratory effort as an indicator of diaphragmatic strength helps in the assessment of patients with respiratory muscle weakness (Hillman et al. 1990; Zakynthinos and Roussos 2005). Trans-diaphragmatic pressure is the difference between intra-abdominal (P_{abd}) and intra-pleural (P_{pl}) pressure (Hubmayr et al.1990). However, the measurement of Pa_{bd} and P_{pl} is difficult under clinical conditions and therefore, various methods have been proposed for the measurement of P_{di}. Thus, Pa_{bd} and P_{pl} are measured in the stomach (Pg_{ast}) and in the oesophagus (Po_{es}), respectively (Benditt 2005). In an attempt to obtain the maximum deflections of

P_{gast}, P_{oes}, and P_{di} during a respiratory cycle, patients are allowed to breath while the upper airway is fully occluded in a clinical setting. As a result, during inspiration the maximum increase in Pgast, and the maximum decrease in P_{pl} (detected as maximum decrease in P_{oes}) will be observed, and the maximum deflection of P_{di} could be calculated. Maximum deflections of P_{gast}, P_{oes}, and P_{di} during a respiratory cycle are obtained with a special technique, which is called the Mueller's maneuver. In human medicine, the Mueller's maneuver is an attempt of inspiration with closed mouth and nose (or the glottis). A modified technique for the application of Mueller's maneuver has already been described in dogs under anesthesia in clinical conditions (Pavlidou et al. 2014).

To our knowledge, there is no published study in dogs on the effect of methylxanthines on a non-fatigued diaphragm. The aim of this study was to investigate the effect of aminophylline on P_{di} in isoflurane anesthetized male dogs. Our hypothesis was that the administration of aminophylline during anaesthesia might increase diaphragmatic contractility in dogs.

MATERIALS AND METHODS

For this prospective, randomized, non-blinded clinical study, approval from the Ethics Committee of Aristotle University of Thessaloniki was obtained. Each owner was informed in detail about the study protocol and a signed written consent was taken. The study population was animals admitted to the Companion Animal Clinic of Aristotle University of Thessaloniki for castration. Exclusion criteria were status ASA 3 or higher, any active respiratory disease, a history of a chronic respiratory problem, and anticipated necessity to apply intermittent positive pressure ventilation (IPPV) during surgery.

All the animals underwent only castration without any other abdominal/thoracic surgical procedure so as to avoid any effects of the atmospheric pressure on the open cavities and of the surgical manipulations on the P_{oes} and P_{gast}. Castration was performed in left or right lateral recumbency, in an attempt to minimize the effect of body position on the P_{oes} and P_{gast}. Obese animals were excluded from the study since

it has been shown that obesity may decrease diaphragmatic contractility in humans (Ora et al. 2011). The evaluation of the obesity was based on the body condition scoring system (1-5). Obese animals were scored as 4 or 5. Furthermore, it has been shown that in very small or large breed dogs, the introduction and proper placement of the balloon catheter into the stomach is difficult and the P_{gast} measurement is inaccurate (Pavlidou et al. 2014). Therefore, medium size dogs were used in this study.

The animals were hospitalized in the Clinic for at least one day before surgery. Pre-anesthetic evaluation included physical examination, complete blood count, serum biochemistry (determinations of albumin, urea, creatinine, potassium and glucose concentrations, alkaline phosphatase/ALP and alanine transaminase/ALT activities) and thoracic /abdominal radiographs. The animals were fasted for eight hours with dry food and they had free access to water for up to two hours before premedication.

Animal preparation

Eighteen, healthy, male, client-owned dogs, 1-10 (1.7±1.3) years (mean ± standard deviation) old and 5-30 (15.5 ± 6.5) kg body weight were enrolled. All animals were premedicated with dexmedetomidine (Dexdomitor, Pfizer Hellas, Athens, Greece) at 175μg/m2 and morphine (Morphine sulfate, Famar SA, Athens, Greece) at 0.1 mg/kg intramuscularly. Twenty minutes later the cephalic vein was catheterized and the administration of Lactated Ringer's solution (LR's, Vioser, Trikala, Greece) at 10 ml/kg/h intravenously (IV) commenced. Anesthesia was induced with propofol (Propofol MCT/LCT, Fresenius, Fresenius Kabi Greece) to effect. In particular, an initial dose of 2 mg/kg was given IV and then incremental doses of 1 mg/kg were injected until endotracheal intubation could easily be performed. Anesthesia was maintained with isoflurane (Isoflurane, Bayer, Leverkusen, Germany) in oxygen. All the animals were breathing spontaneously. Fresh gas flow was delivered at 1.5 L/min through a semiclosed circle rebreathing system. Carprofen (Rimadyl, Pfizer Hellas, Athens, Greece) was administered just after intubation at 2 mg/kg IV. The animals were randomly allocated into two groups. In the animals of group A, aminophylline (Aminophylline,

Demo, Athens, Greece) was administered as an intravenous bolus at 4 mg/kg just after induction, whereas in the animals of group C, no aminophylline was given.
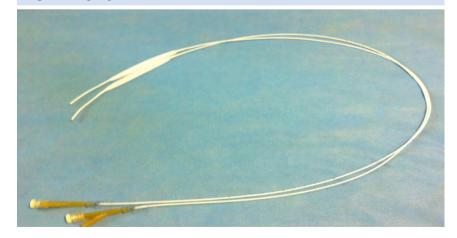
Heart rate (HR), respiratory rate (RR), mean arterial blood pressure (MAP) (measured non-invasively using oscillometry with the cuff placed around the forelimb), end-tidal carbon dioxide partial pressure (PE'CO$_2$) and end-tidal isoflurane fraction (FE'iso) were constantly monitored (Datex-Ohmeda S/5, GE Healthcare, Helsinki, Finland) and recorded every 5 minutes. In case of a PE'CO$_2$ higher than 8.5 kPa, artificial ventilation was applied (McDonell and Kerr 2007) and the measurement of P$_{di}$ was cancelled. During surgery, all the animals were placed in lateral recumbency, left or right.

Clinical signs along with electronic monitoring readings were used in order to assess the adequacy of anesthetic depth. Such signs were lack of reflexes, presence of adequate muscle relaxation, and a lack of physiological response to surgical stimulation characterized by less than 10 % change in HR, RR and MAP during surgical stimulation.

Trans-diaphragmatic pressure measurement

When a surgical plane of anesthesia was achieved, two 90 cm long oesophageal balloon catheters (Esophageal Balloon Catheter Set, CooperSurgical Company, Trumbull, USA) (Figure 1) with guide wires were used for the measurements of P$_{oes}$ and P$_{gast}$. The balloon of the first catheter was introduced into the stomach for the measurement of P$_{gast}$ and

Fig 1. Oesophageal balloon catheter

the distal end of the second catheter was positioned in the mid-third of the oesophagus for the measurement of P$_{oes}$. The correct positioning of the two catheters was confirmed by the observation of the respective pressure tracings on the computer screen: a positive deflection (increase in pressure) during each inspiration was an indication of the correct intra-gastric position of the balloon of the catheter, whereas a negative deflection (decrease in pressure) during each inspiration, confirmed placement of the balloon of the second catheter into the oesophagus. The proximal end of each catheter was connected to a pressure-transducer and then to a recording device (Pressure Monitoring system Buzzer-II, Michael Roehrich, Austria). The catheters were secured in place by fixing them with an adhesive tape to the endotracheal tube. Following the removal of the guide wires, the balloons were inflated with 0.5-1 ml of air. All the pressure measurements were saved with a sampling rate of 10 Hz.

For the measurement of P$_{di}$, it was necessary to obtain the maximum deflections of Pgast (ΔP_{gast}) and P$_{oes}$ (ΔP_{oes}) during a respiratory cycle. To achieve this, the endotracheal tube was disconnected from the anesthetic circuit and tightly closed with a thumb after the end of expiration, so that the animal was forced to breathe against a completely closed airway (Laporta and Grassino 1985; Pavlidou et al. 2014) (modified Mueller's maneuver). P$_{gast}$ and P$_{oes}$ were assessed 3 times during a period of 60 minutes independently of the duration of the surgery. In order to calculate P$_{di}$, the Mueller's maneuver of a single obstruction was performed three separate times on each animal, with a 30-minute interval between each measurement (one just after the anesthetic induction, one just after the start of the surgery and one at the end of the 60 minutes period). The balloon catheters were removed after the end of the 60-minute period independently of the duration of surgery.

All the data from the recording device were saved in a spread sheet. Then, they were analyzed with a signal analysis software (Qtiplot, MicroCal, Northampton,

Massachusetts, USA). From the data for each pressure (gastric or oesophageal), a positive curve for gastric pressure and a negative curve for oesophageal pressure respectively, were drawn. The baseline of gastric and oesophageal curves was zeroed. In every set of measurements, the P_{di} value was calculated (difference between P_{gast} and P_{oes}). The area under the curve (AUC) for each variable was calculated, using the trapezoid method (Matthews et al. 1990). The AUCs were then standardized by the duration of measurements (60 min).

Statistical analysis was performed with a statistical software (SPSS 19, IBM company, Illinois, USA). AUCst from all variables were evaluated for normality using the Shapiro-Wilk test, and for difference of means with the t-test. $p < 0.05$ was considered to be statistically significant.

RESULTS

The two groups were homogenous regarding the age (p=0.450) and the weight (p=0.864) of the animals. The mean values of P_{di} as well as of all measured hemodynamic and respiratory parameters are shown in Table 1.

The mean±standard deviation of AUC of P_{di} was 14.42±4.87 mmHg (1.87±0.63 kPa) in group A and 11.62±3.54 mmHg (1.51±0.46 kPa) in group C, with the difference being statistically non-significant (p=0.182). Moreover, the mean±standard deviation of AUC of PE'CO_2 was higher in group A (6.8 ± 0.8 kPa) than in group C (6.5±0.3 kPa) without a statistically significant difference (p=0.316). Regarding AUC of FE'iso, it was not significantly (p=0.181) different in group A (1.4±0.2 %) than in group C (1.6±0.2 %).

DISCUSSION

The present clinical study was designed to investigate the effect of aminophylline on diaphragmatic contractility in dogs under anesthesia. The evaluation of diaphragmatic contractility was based on the modified technique for P_{di} measurement with balloon catheters (Pavlidou et al. 2014). P_{di} value in control group was 11.62±3.54 mmHg and this finding is in accor-

dance with the P_{di} reference published values in our previous study (Pavlidou et al. 2013). Regarding the anesthetic protocol, it was the same with the protocol (premedication, induction, maintenance) that has been used in the other two published clinical studies about P_{di} (Pavlidou et al. 2013, Pavlidou et al. 2014). This anesthetic protocol seems to have a weak effect on diaphragmatic contractility.

In both human and veterinary medicine, the main indication for use of methylxanthines is their bronchodilator action, especially in patients with respiratory and cardiovascular problems (Aubier et al. 1981; Plumb 2002). Aminophylline enhances diaphragmatic contractility and increases P_{di} in experimental studies (Aubier 1981; Aubier et al. 1983b; Aubier

Table 1. Mean ± standard deviation (SD) and p values of P_{di}, haemodynamic and respiratory variables in the two groups (group A, aminophylline-group C, control).

Variable	Statistic	Group	
		A	C
P_{di} (mmHg) (p=0.182)	N	9	9
	Mean	14.4	11.6
	SD	4.8	3.5
HR (min⁻¹) (p=0.719)	N	9	9
	Mean	82.0	83.6
	SD	8	10.5
RR (min⁻¹) (p=0.956)	N	9	9
	Mean	9.7	9.6
	SD	5.2	5.3
MAP (mmHg) (p=0.124)	N	9	9
	Mean	88.6	75.7
	SD	15.3	9.8
PE'CO_2 (kPa) (p=0.316)	N	9	9
	Mean	6.8	6.5
	SD	0.8	0.3
FE'iso (%) (p=0.181)	N	7	8
	Mean	1.4	1.6
	SD	0.2	0.2

1985; Jagers et al. 2009). The exact mechanism by which aminophylline improves diaphragmatic contractility is not yet known. It has been suggested that methylxanthines (theophylline and aminophylline) induce their therapeutic effects by inhibiting phosphodiesterase, which catalyzes the hydrolysis of cyclic AMP (Mazza 1982). However, this hypothesis was questioned and other theories, like prostaglandin inhibition and alteration of calcium movement across the cell membrane, have been proposed. The transfer of intracellular calcium may be responsible for the direct action of aminophylline on skeletal muscles (Aubier et al. 1981; Aubier et al. 1983b; Aubier 1985). Another possible mechanism of action is the elongation of the diaphragmatic fibers. Methylxanthines have been shown to strengthen and elongate the fibers of skeletal muscles (especially of the diaphragm) after the electrical stimulation of these muscles (Aubier et al. 1983b).

In an experimental study, various ventilatory parameters, P_{di} and electromyographic activity in awake canines were measured. It was found that aminophylline, in the usual dose rate for humans (a loading dose of 7 mg/kg followed by a continuous rate infusion at 1.5 mg/kg/h), increases ventilation and contractility of the respiratory muscles (Jagers et al. 2009).

In humans, methylxanthines have been used in many clinical trials and mainly in patients with respiratory disease (Aubier et al. 1981; Eldridge et al. 1983; Levy et al. 1990; Moxham et al. 1985; Murciano et al. 1984; Murciano et al. 1989; Sigrist et al. 1982). Most of the trials support the inotropic effect of aminophylline on respiratory muscles and especially on diaphragm (Aubier et al. 1981). Additionally, different doses of aminophylline have also been used in humans in order to investigate its effect on diaphragmatic contractility and P_{di} (Levy et al. 1990).

In dogs, the therapeutic dose of aminophylline is 6-11 mg/kg per os, intramuscularly, or intravenously (Plumb 2002). Methylxanthines have a low therapeutic index and therefore, the dosage should be calculated carefully. The main side effects of methylxanthines in dogs and cats are vomiting, nausea, insomnia, diarrhea, polyphagia, polyuria and poly-

dipsia. Moreover, aminophylline can affect the cardiovascular system causing tachycardia, hypotension, arrhythmias and acute respiratory failure, especially when it is injected intravenously and in non-anesthetized patients (Plumb 2002). A dose of 4 mg/kg as an intravenous bolus of aminophylline was used in this clinical study. This is a low dose but it was chosen so that any adverse effects of aminophylline would be eliminated, as this was a clinical investigation. Moreover, the clinical study was not fully blinded so as the anesthetist was aware of the chosen group in order to recognize and treat any side effects of aminophylline.

In this study, P_{di} was higher in the aminophylline group(14.4 mmHg) than in the control group(11.6 mmHg), although the difference was statistically non-significant (p=0.182, achieved power, i.e. 1-β error probability = 0.378). If there is no true effect of aminophylline in P_{di}, our results suggest that there is an 18.2% probability of this difference not being a random effect. Although there is a high probability of error if we accept a true effect of aminophylline, this may be of clinical importance, especially in compromised animals. Moreover, there was also a statistically non-significant difference between the two groups with regards to $PE'CO_2$(p=0.316), although $PE'CO_2$ was higher in group A than in group C. In animal studies, it has been shown that minute ventilation increased with increasing the concentration of aminophylline in plasma in conscious dogs. This phenomenon has been explained by the direct effect of the drug on the respiratory centers or by increasing the sensitivity of these centers to the carbon dioxide or hypoxia (Aubier et al. 1983a).

Aminophylline can cause tachycardia and hypotension via positive inotropic and vasodilatory actions (Gayan-Ramirez et al. 1994; Rutherford et al. 1981). However, in the present clinical study, the mean arterial pressure was higher in the aminophylline group (A) than in the control group (C), and the heart rate was higher in group C than in group A, although both differences were statistically non-significant. Hypotension, as an adverse effect of aminophylline administration may not have been caused in this clinical study, because of the low dose administered (4 mg/kg), as hypotension was observed when ami-

nophylline was administered at higher doses (over 7 mg/kg) (Mazza 1982; Pearl et al. 1984).

CONCLUSIONS

Although our sample size and the used dose rate of aminophylline were small, there seems to be a tendency of aminophylline to increase diaphragmatic contractility, in dogs under general anesthesia. The clinical importance of this finding has to be evaluated further in a larger number of clinical cases. Moreover, it would be of interest to investigate the effect of aminophylline administration on diminishing or even cancelling the depressant effects of other anesthetic or analgesic drugs on P_{di} intra-operatively especially in patients with respiratory disease.

REFERENCES

Aubier M, De Troyer A, Sampson M, Macklem PT, Roussos C (1981) Aminophylline improves diaphragmatic contractility. N Engl J Med 305:249-252.

Aubier M, Murciano D, Viires N (1983a) Increased ventilation caused by improved diaphragmatic efficiency during aminophylline infusion. Am Rev Respir Dis 127:148-154.

Aubier M, Murciano D, Viires N, Lecocguic Y, Pariente R (1983b) Diaphragmatic contractility enhanced by aminophylline: role of extracellular calcium. J Appl Physiol 54:460-464.

Aubier M, Roussos C (1985) Effect of theophylline on respiratory muscle function. Chest 88:91-97.

Benditt JO (2005) Esophageal and gastric pressure measurements. Respir Care 50:68-75.

Eldridge FL, Millhorn DE, Waldrop TG, Kiley JP (1983) Mechanism of respiratory effects of methylxanthines. Respir Physiol 53:239-261.

Gayan-Ramirez G, Palecek F, Chen Y, Janssens S, Decramer M (1994) Inotropic effects of aminophylline on canine diaphragm are enhanced by hyperinflation. J Appl Physiol 76:39-44.

Hillman DR, Markos J, Finucane KE (1990) Effect of abdominal compression on maximum transdiaphragmatic pressure. J Appl Physiol 68:2296-2304.

Hubmayr RD, Sprung J, Nelson S (1990) Determinants of transdiaphragmatic pressure in dogs. J Appl Physiol 69:2050-2056.

Jagers JV, Hawes HG, Easton PA (2009) Aminophylline increases ventilation and diaphragm contractility in awake canines. Respir Physiol Neurobiol 167:273-280.

Kochi T, Ide T, Mizuguchi T, Nishino T (1992) Halothane does not depress contractile function of fresh or fatigued diaphragm in pentobarbitone-anaesthetized dogs. Br J Anaesth 68:562-566.

Laporta D, Grassino A (1985) Assessment of transdiaphragmatic pressure in humans. J Appl Physiol 58:1469-1476.

Levy RD, Nava S, Gibbons L, Bellemare F (1990) Aminophylline and human diaphragm strength in vivo. J Appl Physiol 68:2591-2596.

Matthews JN, Altman DG, Campbell MJ, Royston P (1990) Analysis of serial measurements inmedical research. Br Med J 300:230-235.

Mazza JA (1982) Xanthines in respiratory diseases. Can Fam Physician 28:1799-1803.

McDonell WN, Kerr CL (2007) Respiratory System. In: Lumb & Jones Veterinary Anaesthesia and Analgesia. 4th ed, Blackwell, Iowa: pp 117-151.

Moxham J, Miller J, Wiles CM, Moris AJ, Green M (1985) Effect of aminophylline on the human diaphragm. Thorax 40:288-292.

Murciano D, Aubier M, Lecocguic Y (1984) Effects of theophylline on diaphragmatic strength and fatigue in patients with chronic obstructive pulmonary disease. N Engl J Med 311:349-353.

Murciano D, Auclair MH, Pariente R, Aubier M (1989) A randomized, controlled trial of theophylline in patients with severe chronic obstructive pulmonary disease. N Engl J Med 320:1521-1525.

Nishina K, Mikawa K, Kodama S, Kawaga T, Uesugi T, Obara H (2003) The effects of enflurane, isoflurane, and intravenous anesthetics on rat diaphragmatic function and fatigability. Anesth Analg 96:1674-1678.

Ora J, Laveneziana P, Wadell K, Preston M, Webb KA, O'Donnell DE (2011) Effect of obesity on respiratory mechanics during rest and exercise in COPD. J Appl Physiol 111:10-19.

Pavlidou K, Savvas I, MoensY, Vasilakos D, Raptopoulos D (2013) The effect of four anaesthetic protocols for maintenance of anaesthesia on trans-diaphragmatic pressure in dogs. Plos One 8 (10)doi:10.1371/journal.pone.007534.

Pavlidou K, Savvas I, Moens Y, Vasilakos D, Raptopoulos D (2014) A minimally invasive method for clinical trans-diaphragmatic pressure measurement in anaesthetized dogs. Vet Anaesth Analg41:278-283.

Pearl RG, Rosenthal MH, Murad F, Ashton JP (1984) Aminophylline potentiates sodium nitroprusside-induced hypotension in the dog. Anesthesiology 61:712-715.

Plumb DC (2002) Aminophylline/Theophylline. In: Veterinary Drug Handbook. 4th ed,Blackwell, Iowa, pp 52-55.

Rutherford JD, Vatner SF, Braunwald E (1981) Effects and mechanism of action of aminophylline on cardiac function and regional blood flow distribution in conscious dogs. Circulation 63:378-387.

Sigrist S,Thomas D, Howell S, Roussos C (1982) The effect of aminophylline on inspiratory muscle contractility. Am Rev Respir Dis 126:46-50.

Wanke T, Merkle M, Zifko U, Formanek D, Lahrmann H, Grisold W, Zwick H (1994) The effect of aminophylline on the force-length characteristics of the diaphragm. Am J Respir Crit Care Med 149:1545-1549.

Zakynthinos S, Roussos C (2005) Respiratory muscle fatigue. In: Physiologic Basis Of Respiratory Disease. 1st ed, Hamilton, BC Decker, Ontario, pp 289-307.

Zhang XJ, Yu G, Wen XH, Lin ZC, Yang FQ, Zheng ZG, Chen RC, Zhong NS (2009) Effect of propofol on twitch diaphragmatic pressure evoked by cervical magnetic stimulation in patients. Br J Anaesth 102:61-64.