



Volumetric capnography curves as lung function test to confirm bronchoconstriction after carbachol challenge in sedated dogs

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ABSTRACT

This study investigated volumetric capnography (VC) in detecting airway responsiveness following airway challenge using carbachol in seven sedated dogs via face mask. Nebulised saline was administered, followed by increasing concentrations of nebulised carbachol until airflow limitation occurred (EP). Dead space (DS) variables and shape indices of the VC curve were calculated automatically after entering arterial carbon dioxide tension. Airway DS, airway DS to tidal volume (V_T) ratio and the intercept of slope 2 of the VC curve decreased significantly at EP by 10%, 13% and 16%, respectively, minute ventilation, V_T and alveolar DS increased significantly at EP by 49%, 22% and 200%, respectively. We conclude that VC and derived indices may be used to verify a reaction to airway challenge caused by carbachol in sedated dogs.

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1. Introduction

Lung function tests are well established procedures in human medicine. Cholinergic agonists are used to challenge the airways, leading to airflow limitation due to bronchoconstriction and the assessment of non-specific airway responsiveness (Crapo et al., 2000). Non-invasive, clinically applicable and sensitive pulmonary function tests used to diagnose subclinical respiratory tract diseases in dogs remain challenging due to the requirements of expensive equipment and the cooperation of the patient or general anesthesia (Dye and Costa, 2004). Volumetric capnography (VC) has been introduced recently to human medicine for lung function testing with a focus on distal airways (Olsson et al., 1999; Steiß et al., 2008). This test measures airway dead space based on Fowler's method and calculates alveolar and physiologic dead space using the Bohr-Enghoff equation (Enghoff, 1938; Fowler, 1948). During lung function tests bronchoconstriction can be diagnosed by a decrease in airway dead space and an increase in alveolar dead space leaving the physiologic dead space unchanged (Olsson et al., 1999). Volumetric capnography requires a capnograph and a pneumotachograph. The expired CO_2 is measured and plotted against the exhaled volume (Fletcher et al., 1981). Respiratory variables related to pulmonary dead space and specific VC indices can be recorded and evaluated in real time. Volumetric capnography has

been used in veterinary medicine to diagnose obstructive airway diseases in horses (Herholz et al., 2003). It has been compared to the gold standard by using the mixing box technique to evaluate Bohr-Enghoff dead space in dogs (Mosing et al., 2010) and to detect bronchoconstriction in anaesthetised dogs (Mosing et al., 2011).

The aim of this study was to investigate the value of VC and derived indices in detecting airflow limitation following carbachol challenge in sedated dogs via a face mask. The hypothesis was that non-invasively derived volumetric capnography indices can be used to verify bronchoconstriction at the time point of first visible clinical signs.

Preliminary results presented in this paper have been published in abstract form (Scheffzek et al., 2007).

2. Materials and methods

2.1. Animals

The study protocol was discussed and approved by the institutional ethics committee of the Veterinary University Vienna and also gained government approval (GZ:68-205/0015-57 BrGT/2007). Included in the study were seven male-castrated beagle dogs aged 23.1 ± 12.2 months with a weight of 15.9 ± 2.9 kg (mean \pm standard deviation). All dogs were vaccinated and dewormed on a regular basis. Based on clinical examination, thoracic

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radiographs and routine blood examination, the animals were considered healthy when entering the study.

2.2. Face mask

A custom made face mask was used. The outer shell of the mask was made of synthetic material. The shape of the shell was adapted to mesocephalic dogs. An inflatable latex cuff lined the mask on the inside of the shell. This cuff was inflated after applying the mask to the muzzle of the dog in order to reduce mechanical dead space. A latex strap circumferentially attached to the muzzle of the animal, ensured tight contact between the cuff and the head of the dog. A similar mask for dogs has been previously described (Amis et al., 1982).

2.3. Respiratory monitor and VC variables

The VC unit (NICO₂[®], Respirationics Inc., Murrysville, Pennsylvania) consists of a fixed-orifice pneumotachograph combined with a mainstream infrared capnograph. For measurement of respiratory parameters the sensors of the VC unit were positioned between the face mask and the breathing circuit. Dedicated software (AnalysisPlus![®]6.0, Novamatrix Medical Systems Inc., Wallingford, Connecticut) installed on a laptop computer displayed, recorded and stored all respiratory data for on-line and off-line analysis. The software automatically generated a VC curve and calculated VC variables on a breath-by-breath basis.

The VC curve has three phases: phase 1 represents the exhalation of CO₂-free gas from the conducting airways; phase 2 is defined as the S-shaped upswing of the curve where CO₂-free gas from the conducting airways and CO₂-rich alveolar gas mixes; phase 3 represents the alveolar plateau containing CO₂-rich alveolar gas (Fig. 1A). Physiological dead space ($V_{DS\ phys}$) is automatically calculated by the software (Mosing et al., 2010). The calculation is based on mixed expired CO₂ and arterial partial pressure of CO₂ (P_{aCO_2}). Both variables are entered into the Bohr-Enghoff equation (Enghoff, 1938; Mosing et al., 2010).

Airway dead space ($V_{DS\ aw}$) is computed according to Fowler's method (Fowler, 1948) (Fig. 1A). Alveolar dead space ($V_{DS\ alv}$) is obtained by subtracting $V_{DS\ aw}$ from $V_{DS\ phys}$. Phase 2 and 3 are characterised by their slopes and the corresponding regression lines (slope 2 and slope 3). An extension of the regression lines results in the intercept of slope 2 and slope 3 with the y-axis (InterceptY2 and InterceptY3) (Fig. 1).

2.4. Experimental protocol

At the beginning of each experiment the capnograph of the VC unit was calibrated according to the guidelines of the manufacturer. Following catheterisation of a cephalic vein each dog was sedated with 0.03 mg/kg ($n = 4$) to 0.05 mg/kg ($n = 3$) acepromazine, dependent on the level of excitement of the dog, and 0.01 mg/kg buprenorphine intravenously (IV). Twenty minutes later the auricular artery was catheterised for arterial blood sampling (22 G, Braunüle, Braun, Melsungen, Germany) and invasive monitoring of the mean arterial pressure (MAP) (Combitrans Monitoring-set arteriell, Braun, Melsungen, Germany) with the pressure transducer zeroed to atmosphere and levelled at the height of the manubrium. The dogs were placed on electrical heating pads in right lateral recumbency. The body temperature was monitored using a rectal probe. An electrocardiograph (ECG; adhesive ECG electrodes at both metacarpal and left metatarsal pad) was attached to monitor for arrhythmias. Lactated Ringer's solution was administered IV at a rate of 5 mL/kg/h throughout the study period using an infusion pump. Recording of all respiratory variables, HR (taken from the arterial pressure trace and the ECG) and MAP

started approximately 45 min after the sedative drugs had been injected (Datex AS5 Monitor, Datex Ohmeda, Duisburg, Germany).

For the airway challenge with carbachol, the face mask was applied to the muzzle of the dog and connected to a non-rebreathing system (Parallel Lack, Intersurgical Ltd., Wokingham, UK). An oxygen-air mixture containing 40% oxygen at a fresh gas flow of 4 L/min was administered. The combined CO₂/flow-sensor of the VC unit was positioned between the face mask and the breathing system (Fig. 2). Before starting the airway challenge, the latex cuff of the mask was inflated until flow-volume-loops (visualisation of the difference/similarity between inspiratory and expiratory tidal volume by return of the loop to the starting point) indicated the absence of leaks in the system.

The endpoint (EP) was defined as the carbachol challenge when a clinical reaction occurred. A clinical reaction was either (1) clinical signs suggesting airflow limitation as agreed by all observers or (2) other effects of carbachol administration (hypotension, piloerection, urination, defecation, salivation, and vomiting) or (3) a decrease in heart rate from the pre-challenge value by 20%. The appearance of airflow limitation was independently scored by three experienced, non-blinded veterinarians observing the breathing pattern of the dog under investigation. The observers were interpreting changes in breathing patterns namely paradoxical breathing and increased expiratory effort visible as prolonged expiration with increased abdominal muscle contractions.

2.5. Nebulisation of carbachol

Bronchoconstriction was provoked by inhalation of nebulised carbachol solutions in increasing concentrations. The aerosol was generated by an electronically driven nebuliser using vibrating-mesh-technology (Aeroneb[®]Pro, Aerogen Ltd., Dangan, Ireland). The nebuliser delivered particles with a median diameter of 2.1 μ m and had a consumption of >0.3 mL/min fluid. The device was integrated in the inspiratory limb of the breathing system, close to the y-piece. Prior to this study the performance of the nebuliser was tested gravimetrically in vitro at different flow rates. According to these measurements, the flow in the breathing system was chosen to be 4 L/min. Based on a 1% stock solution of carbachol (Carbachol, FlukaChemie GmbH, Buchs, Switzerland), 0.005%, 0.01%, 0.025%, 0.05%, 0.075% and 0.1% solutions using sterile saline as the diluents were prepared. The diluted solutions were prepared before each experiment and used within 3 h.

2.6. Airway challenge

Baseline measurements were obtained before the first nebulisation. A control measurement was performed with nebulisation of 0.9% saline (inhalant vehicle). Thereafter, airway challenge was performed by inhalation of stepwise increasing concentrations of nebulised carbachol starting with 0.005%. Each concentration was administered for 3 min. The next airway challenge was started after the main outcome variable, $V_{DS\ aw}$, had returned to baseline level for 1 min. Mean time between airway challenges was 8.3 min. Data was collected during the 3 min preceding and the 4 min following each nebulisation period. Arterial blood samples were drawn from the ear catheter into a heparinised syringe at baseline and the minute before and after each nebulisation and immediately analysed using temperature corrected values (Synthesis 25, Instrumentation Laboratory, Vienna, Austria; 3-level quality control performed daily). Sampling time and values of P_{aCO_2} for each blood sample were entered into the VC unit for calculation of VC indices. In the case of a visible clinical reaction (EP), the experiment was stopped. A time scale is shown in Fig. 3.

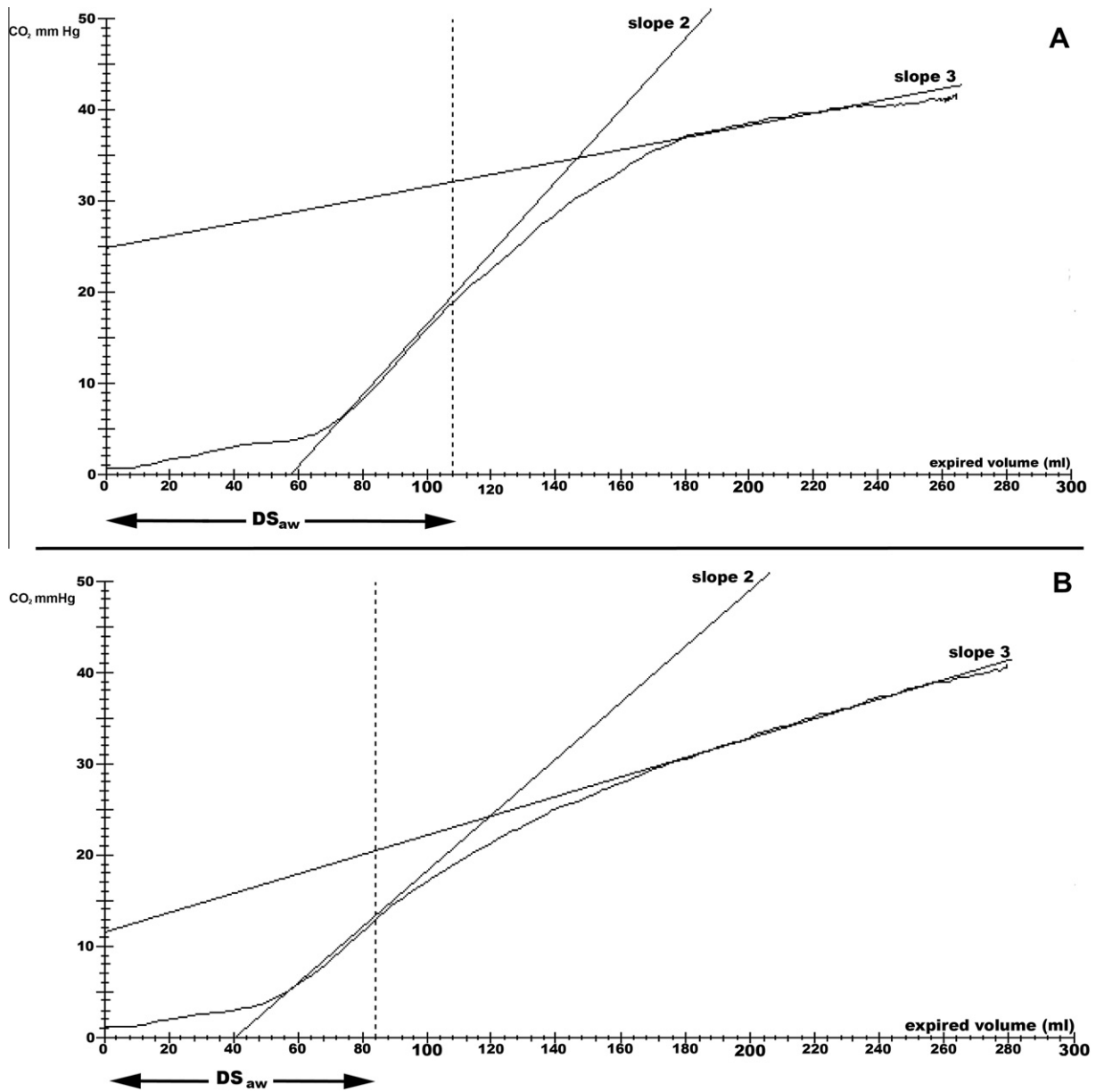


Fig. 1. (A) Volumetric capnography (VC) curve of a dog under resting state. (B) Changes in the VC curve after nebulisation of carbachol leading to airflow limitation. The intercept of the intersected line with the x-axis represent the volume of the airway dead space (DS_{aw}).

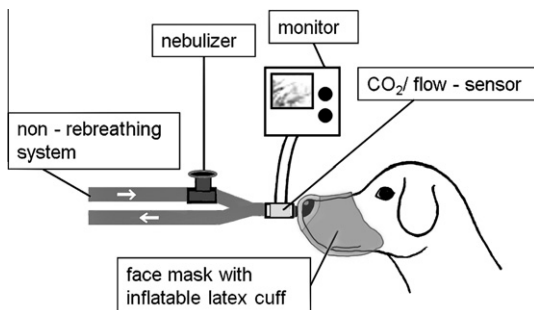


Fig. 2. Graphical illustration of the study set-up. The face mask was attached to the muzzle of the dog. The pneumotachograph and mainstream capnograph were connected to the rostral opening of the mask (CO_2 /flow-sensor). The nebulizer was integrated into the inspiratory limb of the non-rebreathing system. White arrows show the direction of the inspiratory flow towards the dog and the expiratory flow towards the reservoir bag and distal end of the system.

2.7. Off-line analysis of data

The following parameters were selected for off-line analysis:

- (1) For parameters recorded in intervals of 1 min (minute ventilation, RR, V_T , $V_{DS_{aw}}$, $V_{DS_{aw}}/V_T$) the values for the 3 min before and 4 min after nebulisation were obtained.
- (2) For the parameters calculated using P_{aCO_2} ($V_{DS_{phys}}$, $V_{DS_{phys}}/V_T$ and $V_{DS_{alv}}$) the variables were obtained at the time of blood sampling (1 min before and 1 min after the nebulisation period).
- (3) The InterceptY2 and InterceptY3 were recorded for each breath during 3 min before and 4 min after the nebulisation period. Only breaths where the software calculated values for intercepts and those without artefacts in the VC graph were included in the analysis.

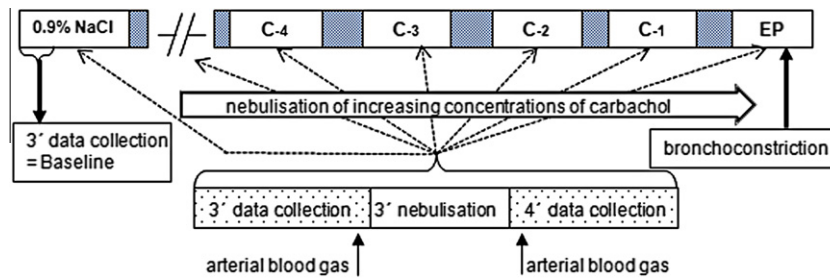


Fig. 3. Time scale and different nebulisation periods of carbachol C₋₄–C₋₁ (nebulisation periods of different carbachol concentrations), EP (end point, when clinical signs of airflow limitation were visible).

Table 1

Overview of the nebulisation steps in seven dogs: BL (baseline before nebulisation of saline), C₋₅–C₋₁ (nebulisation periods of carbachol prior to EP), EP (end point, when clinical signs of airflow limitation occurred).

Dog	BL	C ₋₅ (%)	C ₋₄ (%)	C ₋₃ (%)	C ₋₂ (%)	C ₋₁ (%)	EP (%)
1	Saline		0.005	0.01	0.025	0.05	0.075
2	Saline		0.005	0.01	0.025	0.05	0.075
3	Saline	0.005	0.01	0.025	0.05	0.075	0.1
4	Saline	0.005	0.01	0.025	0.05	0.075	0.1
5	Saline			0.005	0.01	0.025	0.05
6	Saline	0.005	0.01	0.025	0.05	0.075	0.1
7	Panting	Panting	Panting	Panting	Panting	Panting	Panting

A high inter-individual difference in the onset of detectable signs within the 4 min after each carbachol challenge was observed in all dogs. Therefore, the highest (V_T , minute ventilation) or lowest (InterceptY2, InterceptY3, $V_{DS\text{ aw}}$, $V_{DS\text{ aw}}/V_T$, and RR) of the averages over 1 min derived within the 4 min after nebulisation were used for statistical analysis.

To obtain the average over the same time period only the data of the last minute before each nebulisation were averaged to obtain the pre-nebulisation value for statistical analysis.

2.8. Statistical analysis

As the dogs needed different carbachol concentrations to get to clinical reaction (EP), the four measurement periods (C₋₄, C₋₃, C₋₂ and C₋₁) prior to EP were included in the statistical analysis (Table 1 and Fig. 3). This approach ensured comparable data between the dogs. Nebulisation periods at low concentrations with less than five dogs were excluded from statistical data analysis (Table 1).

With the exception of HR and MAP, all data are described using median and interquartile ranges and compared using nonparametric tests. For HR and MAP mean \pm SD, parametric tests were used. Overall a $P < 0.05$ was considered significant.

The differences in RR, $V_{DS\text{ aw}}$, $V_{DS\text{ aw}}/V_T$, V_T , minute ventilation, interceptY2 and interceptY3 were evaluated using a Wilcoxon signed rank test (a) before (baseline) and after nebulisation of saline, (b) before and after each nebulisation step and (c) between baseline and nebulisation period that provoked a clinical reaction (EP).

3. Results

One dog was panting throughout the study period and these measurements were thus excluded from the data analysis. The six remaining dogs (age 24.7 ± 12.6 months, body weight 16.3 ± 3 kg) showed clinical signs of airflow limitation during the stepwise challenge. Carbachol concentrations leading to visible airflow limitation agreed by three observers were 0.05% in one dog,

0.075% in two dogs and 0.1% in three dogs (Table 1). At EP, no additional cholinergic effects were detected in any of the dogs. The heart rate and MAP did not significantly change over time.

No significant change was seen following nebulisation of saline in any parameters; therefore all of them were used in statistical analysis. The VC curves of two of the dogs showed a distinct step in phase 2 during three measurement periods. Data recorded during these measurement periods was also excluded from data analysis. Values for the different parameters recorded at each nebulisation period (C₋₄–C₋₁ and EP) are provided in Table 2.

Following variables changes significantly post-nebulisation before EP: tidal volume and minute ventilation increased at C₋₂ and C₋₁, respectively. The airway dead space to V_T ratio decreased significantly after carbachol challenge compared to the pre-nebulisation values at C₋₂ and EP. Physiologic and alveolar dead space increased before and after C₋₁. InterceptY2 increased significantly at C₋₃ and C₋₂.

At EP values minute ventilation, V_T , $V_{DS\text{ phys}}$, $V_{DS\text{ alv}}$ and interceptY2 increased after nebulisation, whereas $V_{DS\text{ aw}}$ and $V_{DS\text{ aw}}/V_T$ decreased significantly when compared to pre-nebulisation values.

No significant changes were seen in RR, $V_{DS\text{ phys}}/V_T$ and InterceptY3 throughout the study period.

When comparing baseline values with those obtained at EP, a significant increase was recorded in $V_{DS\text{ alv}}$, and a significant decrease in $V_{DS\text{ aw}}/V_T$.

The lowest value for arterial partial pressure of oxygen (P_{aO_2}) after nebulisation was 17 kPa (130 mmHg) measured at C₋₁ in one dog. At EP P_{aO_2} ranged between 20 and 29 kPa (152–217 mmHg). Values for P_{aO_2} , P_{aCO_2} and end-tidal CO₂ are shown in Table 3.

4. Discussion

In the present study, the airway responsiveness in dogs was studied through airway challenge and volumetric capnography using a custom made face mask. The aim of the study was to identify VC variables that enable verification of bronchoconstriction at the lowest possible carbachol concentration causing clinical signs of changes in airway diameter. Three non-invasive volumetric capnography indices ($V_{DS\text{ aw}}/V_T$, $V_{DS\text{ aw}}$ and interceptY2) obtained on a breath-by-breath basis were indicating changes in airway diameter when minimal clinical signs were visible. Therefore tests for airway responsiveness using VC might reduce stress for the patient and minimise changes in lung physiology due to early detection of airflow limitation. Clinically not a single VC variable should be evaluated, but all non-invasive indices should be put in context to each other. To confirm the suspicion of bronchoconstriction obtained by the non-invasive variables an increase in the more invasive $V_{DS\text{ alv}}$, requiring arterial blood gas analysis, can be used. An increase in $V_{DS\text{ alv}}$ is a well-described phenomenon in conjunction

Table 2
Median (interquartile range) values for respiratory parameters and variables derived by volumetric capnography before and after nebulisation of carbachol (mean \pm standard deviation).

Variables	Saline		C ₋₄ , n = 5 Carbachol (%) (0.008 \pm 0.002)		C ₋₃ , n = 6 Carbachol (%) (0.067 \pm 0.008)	
	Before (BL)	After	Before	After	Before	After
RR (breaths/min)	11 (8–14)	10 (8.5–11.5)	10 (9–11)	9 (8–10)	10 (7.5–12.5)	9 (7.5–10.5)
MV (L/min)	2.63 (1.32–3.01)	2.63 (1.58–3.68)	2.24 (2.11–2.38)	2.43 (1.98–2.88)	2.48 (1.79–3.18)	2.62 (2.22–3.03)
V _T (mL)	199 (144.5–253.5)	212 (164–260)	222 (167–277)	273 (222.5–323.5)	235 (117.5–352.5)	241 (219–263)
V _{DS aw} (mL)	81 (65.5–96.5)	79 (70–88)	88 (77–99)	74 (67.5–80.5)	89 (75–103)	78 (70–86)
V _{DS aw} /V _T	0.43 (0.4–0.47)	0.40 (0.35–0.45)	0.42 (0.38–0.46)	0.39 (0.36–0.43)	0.40 (0.34–0.46)	0.37 (0.35–0.4)
V _{DS phys} (mL)	91 (77.5–104.5)	100 (86.5–113.5)	100 (90.5–109.5)	95 (81–109)	88 (68–108)	93 (73.5–112.5)
V _{DS phys} /V _T	0.51 (0.45–0.58)	0.48 (0.38–0.58)	0.43 (0.34–0.53)	0.43 (0.38–0.49)	0.52 (0.45–0.6)	0.45 (0.38–0.53)
V _{DS alv} (mL)	13 (9.5–16.5)	9.5 (2–17)	4 (0–12)	10 (5.5–14.5)	12 (6.5–17.5)	15 (5–25)
InterceptY3 (mmHg)	19 (13–25)	24 (13.5–34.5)	24 (18.5–29.5)	21 (15–27)	30 (21.5–38.5)	25 (19.5–30.5)
InterceptY2 (mmHg)	–32 (–38.5–25.5)	–28 (–34 to –22)	–34 (–39.5 to –28.5)	–31 (–35 to –27)	–30 (–34 to –26)	–28 (–31.5 to –24.5)*
	C ₋₂ , n = 6 Carbachol (%) (0.035 \pm 0.016)		C ₋₁ , n = 6 Carbachol (%) (0.058 \pm 0.019)		EP, n = 6 Carbachol (%) (0.083 \pm 0.019)	
	Before	After	Before	After	Before	After
RR (breaths/min)	10 (9–11)	8 (7–9)	10 (9–11)	9 (7.5–10.5)	10 (8–12)	9 (6.5–11.5)
MV (L/min)	2.04 (1.76–2.33)	2.44 (2.27–2.62)	2.17 (2.03–2.32)	3.02 (2.45–3.59)*	2.49 (2.05–2.94)	3.46 (2.86–4.07)*
V _T (mL)	214 (172–256)	282 (244–320)*	205 (168.5–241.5)	255 (201–309)	206 (152.5–259.5)	253 (187–319)*
V _{DS aw} (mL)	76 (70–82)	72 (62.5–81.5)	84 (76.5–91.5)	75 (66–84)	81 (69–93)	73 (63.5–82.5)*
V _{DS aw} /V _T	0.38 (0.36–0.41)	0.33 (0.28–0.39)*	0.38 (0.37–0.4)	0.30 (0.29–0.32)	0.34 (0.31–0.37)	0.30 (0.26–0.35)*#
V _{DS phys} (mL)	88 (72–104)	96 (54.5–83.5)	86 (68.5–103.5)	120 (87.5–152.5)*	85 (65–105)	124 (89.5–158.5)*
V _{DS phys} /V _T	0.44 (0.38–0.51)	0.39 (0.32–0.46)	0.43 (0.37–0.5)	0.43 (0.32–0.55)	0.37 (0.3–0.45)	0.44 (0.32–0.57)
V _{DS alv} (mL)	7 (0–15.5)	16 (12.5–19.5)	11 (2.5–19.5)	28 (6–50)*	14 (5.5–22.5)	42 (13–71)*.#
InterceptY3 (mmHg)	29 (24.5–33.5)	27 (21.5–32.5)	28 (23.5–32.5)	25 (21.5–28.5)	29 (23.5–34.5)	22 (13.5–30.5)
InterceptY2 (mmHg)	–33 (–36 to –30)	–26 (–29.5 to –22.5)*	–30 (–31.5 to –28.5)	–27 (–28 to –26)	–34 (–37 to –31)	–28 (–35.5 to –20.5)*

RR = respiratory rate; MV = minute ventilation; V_T = tidal volume; V_{DS aw} = airway dead space; V_{DS aw}/V_T = airway dead space to tidal volume ratio; V_{DS phys} = physiologic dead space; V_{DS phys}/V_T = physiologic dead space to tidal volume ratio; V_{DS alv} = alveolar dead space; InterceptY3 = intercept of slope 3 with y-axis.

BL (baseline before nebulisation of saline), C₋₁–C₋₄ (nebulisation periods of carbachol prior to EP), EP (end point, when clinical signs of airflow limitation were visible), n (number of dogs).

* Different from before nebulisation at this NP ($P < 0.05$).

Different from baseline ($P < 0.05$).

with bronchoconstriction (Lumb, 2010; Mosing et al., 2011; Niklason et al., 2008; Tusman et al., 2006). This approach might be a good way to use volumetric capnography clinically to evaluate airway responsiveness without the need to provoke severe clinical signs.

The evaluation of airway responsiveness in dogs has been described using barometric whole body plethysmography (BWBP) (Talavera et al., 2006; Hirt et al., 2007, 2008; Bolognin et al., 2009) and traditional measurement of respiratory mechanics that evaluate resistance and dynamic compliance (Hirt et al., 2008). The major limitations for the clinical use of the two methods are the expensive equipment and low sensitivity (BWBP) on one hand and the requirement for general anesthesia (traditional respiratory mechanics) on the other. However, the aim of this study was not to compare established methods of airway responsiveness testing,

but to evaluate the possible usefulness of a new method, namely volumetric capnography.

During carbachol challenge, airway dead space decreased by 10% at EP. As minute ventilation and V_T changed concomitantly the dead space to tidal volume ratios are more meaningful (Fletcher et al., 1981). The airway dead space to V_T ratio (V_{DS aw}/V_T) decreased significantly at EP and at C₋₂. This suggests that the bronchoconstriction caused by carbachol resulted in a real volume reduction in the conducting airways, as recorded by the VC device on a breath-by-breath basis. Changes of V_{DS aw}/V_T occurred before airflow limitation was clinically visible. This finding suggests that VC can be used to detect bronchoconstriction during airway challenge and provides the clinician with the possibility to evaluate the trend before the first visible signs of airflow limitation occur. In the future, this may help to narrow the carbachol concentration

Table 3

Median (interquartile range) values for end-tidal CO₂ (PE'_{CO_2}), arterial partial pressure of oxygen (P_{aO_2}) and CO₂ (P_{aCO_2}) before and after nebulisation of saline and different concentrations of carbachol (mean \pm standard deviation).

Variables	Saline, n = 6		C ₋₄ , n = 5 Carbachol (%) (0.008 \pm 0.002)		C ₋₃ , n = 6 Carbachol (%) (0.067 \pm 0.008)	
	Before (BL)	After	Before	After	Before	After
P_{aO_2} (kPa)	28.7 (28.05–29.35)	28.7 (27.85–29.55)	29.7 (28.4–31.0)	28.9 (28.55–29.25)	29.3 (28.7–29.9)	29.3 (28.9–29.7)
P_{aCO_2} (kPa)	5.6 (5.45–5.75)	5.5 (5.3–5.7)	5.6 (0.21–0.91)	5.6 (5.25–5.95)	5.9 (5.55–6.25)	5.7 (5.45–5.95)
PE'_{CO_2} (kPa)	5.6 (5.2–6.0)	5.6 (5.15–6.05)	5.6 (5.35–5.85)	5.7 (5.45–5.95)	5.6 (5.15–6.05)	5.6 (5.15–6.05)
	C ₋₂ , n = 6 Carbachol (%) (0.035 \pm 0.016)		C ₋₁ , n = 6 Carbachol (%) (0.058 \pm 0.019)		EP, n = 6 Carbachol (%) (0.083 \pm 0.019)	
	Before	After	Before	After	Before	After
P_{aO_2} (kPa)	28.8 (28.15–29.45)	29.1 (28.35–29.85)	28.6 (27.0–30.2)	24.5 (20.1–28.9)	28.7 (27.65–29.75)	20.7 (18.1–23.3)
P_{aCO_2} (kPa)	5.7 (5.5–5.9)	5.6 (5.4–5.8)	5.8 (5.45–6.15)	5.8 (5.4–6.2)	5.8 (5.4–6.2)	5.8 (5.45–6.15)
PE'_{CO_2} (kPa)	5.6 (5.2–6.0)	5.6 (5.15–6.05)	5.6 (5.05–6.15)	5.3 (4.75–5.85)	5.5 (5.05–5.95)	5.6 (4.95–6.25)

BL (baseline before nebulisation of saline), C₋₄–C₋₁ (nebulisation periods of carbachol prior to EP), EP (end point, when clinical signs of airflow limitation occurred), n (number of dogs).

at which the respiratory system starts to react to an airway challenge. The decrease in $V_{DS\text{ aw}}$ aligns with findings in healthy human volunteers during airway challenge where a 19% decrease in $V_{DS\text{ aw}}$ was recorded (Olsson et al., 1999). However, two subjects did not complete the aforementioned study due to severe breathlessness, whereas we were able to show that VC indices are already changing sufficiently to diagnose an airway reaction before or latest at the first clinical signs of bronchoconstriction. So the changes in airway dead space observed in this study appear small, but must be seen under the light of the minimal clinical effects of airflow limitations seen at the same time.

The physiological dead space to V_T ratio ($V_{DS\text{ phys}}/V_T$) remained constant during the carbachol challenge as a consequence of a proportional increase in V_T and $V_{DS\text{ phys}}$. Physiologic dead space is the sum of airway and alveolar dead space. The over proportional increase of $V_{DS\text{ alv}}$ at EP may explain the increase in $V_{DS\text{ phys}}$ in the presence of a decreased $V_{DS\text{ aw}}$. Alveolar dead space ventilation is caused by lung regions which are ventilated but not perfused. An increase in minute ventilation can cause an “alveolar dead space-like effect” as the ratio between ventilation and perfusion is altered meaning an increase in lung regions with a high ventilation–perfusion ratio (Lumb, 2010). Furthermore intrapulmonary shunt and changes in cardiac output often seen during airway challenge using cholinergic drugs can increase alveolar dead space (Niklasen et al., 2008; Tusman et al., 2006). This phenomenon is called shunt-related dead space (Tusman et al., 2006). During bronchoconstriction a reduction in pulmonary capillary blood volume secondary to the high intrathoracic pressure might influence the alveolar dead space measurements (Aliverti et al., 2007). As an increase in V_T was measured after carbachol challenge former explanation might have been the main reason for the increase in alveolar dead space seen in our dogs.

The intercepts of slope 2 and 3 line with the y-axis of the VC curve represent a mathematical magnification of the steepness of slopes. Bronchoconstriction leads to an uneven emptying of different lung regions; this results in areas with low CO₂ emptying first and areas with high CO₂ emptying later in the expiratory period, causing an increase in slope 2 and 3 of the VC curve (Fletcher et al., 1981; Mosing et al., 2011) (Fig. 1). While no significant change was detected in interceptY3, we saw a significant decrease in interceptY2 (equatable with an increase in slope 2) at EP, C₋₂ and C₋₁. This is contradictory to the findings by Mosing et al. (2011), where slope 3 changed significantly after carbachol challenge in dogs, whereas slope 2 remained unchanged. However, different software was used in this paper to evaluate the steepness of the slopes and in both papers only small numbers of animals were

used. Changes in slope 2 and its intercept reveal a change in the positioning of the airway–alveolar interface and therefore is an indicator for asynchronous emptying (Schulz et al., 2004; Tusman et al., 2005).

The technique for airway challenge as described in our study offers several advantages: (1) airway challenge and acquisition of objective data under sedation (preventing the need for general anesthesia and still compiling reproducible data) with minimal cardiovascular compromises, (2) opportunity for oxygen supplementation and prevention of hypoxemia (compared to whole bodyplethysmography, where no additional oxygen can be administered during airway challenge), (3) direct contact between the clinician and the animal allowing for clinical monitoring of the animal (compared to whole bodyplethysmography, where the animal has to sit in a box) and thus the possible avoidance of any sedation, (4) possibility for additional monitoring, such as ECG and measurement of blood pressure (compared to whole bodyplethysmography, where no additional equipment is allowed in the box) and (5) a lack of cholinergic side effects beyond airflow limitation due to significant changes at the point of first clinical signs. Indices from VC curves might even be obtained from unsedated animals, however further studies are necessary to prove this statement and the authors would not recommend the use of carbachol for lung function testing administered over a face mask in a moving dog.

Hypoxemia appears to be a common problem during airway challenge, as P_{aO_2} values of ≤ 80 mmHg while breathing room air are reported (Kowalski et al., 1980; Rodriguez-Roisin et al., 1984). Hypoxemia was avoided through the use of VC and an oxygen enriched inspiratory gas mixture (Fi_{O_2} 0.4). High inspiratory concentrations of oxygen have been shown to cause absorption atelectasis, which would in turn cause changes in VC variables (Fletcher et al., 1981). A Fi_{O_2} of 0.4 did not cause an increase in atelectasis formation (Agarwal et al., 2002). The authors therefore suggest using a Fi_{O_2} of 0.4 for animals with impaired lung function to avoid hypoxemia. Lung volume and respiratory dead spaces can be affected by age, body size and lung disease (Fowler, 1950). All of the dogs included in this study were mature, had nearly the same body size and were free of lung disease.

As no other side effects of carbachol administration were observed throughout the study period, the endpoint in all dogs was the clinical confirmation of airflow limitation. The absence of other cholinergic side effects may have been an effect of the sedative drugs or the lower concentrations used in comparison to previous studies. Cholinergic side effects during carbachol airway challenge were previously observed in dogs sedated with acepromazine and

buprenorphine (Talavera et al., 2006; Hirt et al., 2008). This suggests that the effect of the sedative drugs is unlikely to explain the lack of cholinergic side effects in this study.

One major limitation of this study is the small number of study subjects. Nevertheless, significant changes between before or at the latest at the EP were found for the major respiratory variables (minute ventilation, V_T) and VC indices ($V_{DS\text{ aw}}/V_T$, $V_{DS\text{ phys}}/V_{DS\text{ aw}}$, $V_{DS\text{ alv}}$, and interceptY2). Furthermore the inclusion of a pure saline control group would have been beneficial. Although considered unlikely the pulmonary effects seen in this study could be partially due to repeated saline nebulisation. Data of one dog had to be excluded from the analysis due to the commencement of panting prior to baseline measurements. A high RR does not allow the display of an alveolar plateau of the VC curve. Panting has been described as a possible side effect of carbachol administration (Talavera et al., 2006). This fact may limit the clinical usefulness of the method described. Another limitation for clinical use of the VC method is the necessity of a tightly fitting face mask. A mask for VC recordings should have minimal mechanical dead space and an airtight fit as both factors can influence dead space parameters (Haskins and Patz, 1986). Commercially available face masks for veterinary use do not meet the requirements for VC recordings. The custom made mask in the present study fulfilled all necessary requirements and was designed for the muzzle of a beagle dog. It is likely that for different breeds with varying muzzle sizes and conformation, masks in different sizes and shapes would be required. However, the inflatable latex cuff makes the inner diameter of the mask flexible and guarantees a tight fit in a range of different muzzle sizes.

Each carbachol concentration was administered for 3 min. This time period was chosen in accordance with recent studies on BWBP (Talavera et al., 2006; Hirt et al., 2008). Future studies are required to confirm the usefulness of this time period in the clinical patient. Furthermore, only a direct comparison of the new method using VC with established methods can verify the differences in sensitivity and practicability of VC for clinical lung function tests in dogs.

Volumetric capnography can be used to verify bronchoconstriction during carbachol challenge in dogs (Mosing et al., 2011) and may have the potential to be used as lung function test to evaluate airway responsiveness. With the use of a tightly fitting face mask, general anesthesia is not required. The non-invasive parameters $V_{DS\text{ aw}}/V_T$ and interceptY2 are potential markers for bronchoconstriction as changes occurred before clinical signs were visible. No hypoxemia or other cholinergic side effects were evident in any of the dogs.

Future studies evaluating volumetric capnography with different carbachol nebulization protocols and a larger number of animals from different breeds and sizes are required to validate this technique for assessing airway responsiveness in conscious dogs.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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