



Non-invasive assessment of airway responsiveness in healthy and allergen-sensitised cats by use of barometric whole body plethysmography

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Abstract

This study aimed at determining whether airway responsiveness (AR) tests performed by use of barometric whole body plethysmography (BWBP) were repeatable in cats and to what extent AR was affected by the nebulization protocol used, the age of the animals, the inflammatory status of the airways and prior bronchodilator treatment. Repeatability of AR was tested on two occasions in 30 healthy cats. The concentration of carbachol inducing a 300% increase of the enhanced pause (Penh) – an estimator of airflow limitation – was calculated (C-Penh300) and did not differ significantly between the two tests ($0.035 \pm 0.017\%$ compared to $0.034 \pm 0.016\%$) and was significantly and positively correlated. The comparison between rapidly and slowly increasing carbachol concentrations was performed in ten healthy cats and showed a significantly lower C-Penh300 (%) when slowly increasing concentrations were used ($0.037 \pm 0.013\%$ compared to $0.039 \pm 0.015\%$, $P < 0.05$). A significant age-related increase of C-Penh300 was evidenced by performing AR tests in 15 healthy cats at 12, 18, 24 and 30 months (12 months: $0.026 \pm 0.008\%$, 18 months: $0.031 \pm 0.009\%$, 24 months: $0.038 \pm 0.01\%$, 30 months: $0.043 \pm 0.014\%$, $P < 0.05$). C-Penh300 significantly decreased in 12 *Ascaris suum*-sensitised cats after allergen exposure ($0.026 \pm 0.016\%$ compared to $0.033 \pm 0.016\%$, $P < 0.05$) and was negatively correlated with the granulocyte percentage of bronchoalveolar lavage fluid ($r = -0.36$, $P < 0.01$). Compared with a placebo inhalation, pre-treatment with inhaled salbutamol significantly increased C-Penh300 in four healthy cats ($0.093 \pm 0.021\%$ compared to $0.036 \pm 0.004\%$, $P < 0.05$). This study provides evidence that AR determination by use of BWBP is promising as non-invasive indicator of lower airway inflammation or for monitoring response to bronchodilator treatment.

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1. Introduction

In human medicine, airway hyper-responsiveness has been recognised over the past 30 years as a cardinal fea-

ture of asthma and other pulmonary diseases (O'Byrne, 1998). A variety of methods of measuring airway responsiveness using different agents inducing airway narrowing have been developed and standardised. Assessment of airway responsiveness (AR) is part of the standard investigation in human pulmonary medicine. Increased AR is considered as one manifestation of airway inflammation, as shown in human and animal experimental studies where airway hyper-responsiveness

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induced by allergen inhalation, occupational sensitising agents or ozone is temporally associated with the development of airway inflammation. There is also considerable evidence that a stable stage of airway hyper-responsiveness in asthmatic subjects is associated with persistent airway inflammation, which is of major interest in patients without clinical evidence of airway inflammation such as coughing, wheezing or increased mucus production (O'Byrne, 1998).

In veterinary medicine, AR testing is more frequently performed for research purposes than for clinical diagnostics, which is probably due to the fact that it requires sophisticated equipment and experienced investigators. In large animal species, such as in horses, partly invasive (e.g., necessitating the use of an oesophageal balloon catheter) or non-invasive (e.g., necessitating only a facemask) AR tests have been described and can be performed without or under slight sedation (Gray et al., 1989; Hoffman et al., 2001; Van Erck et al., 2003). In dogs and cats, an invasive AR test can be carried out using ventilatory mechanics, which necessitates general anaesthesia and tracheal intubation (Davis et al., 2005; Dye et al., 1996). Nevertheless, besides measuring AR, these tests further provide interesting information about pulmonary resistance and dynamic compliance, which cannot be obtained using less invasive methods. Among the non-invasive, but less specific pulmonary function tests that might be used in dogs and cats is barometric whole body plethysmography (BWBP) (Hirt et al., 2003; Hoffman et al., 1999; Kirschvink et al., 2005a,b; Talavera et al., 2005). This technique was developed for infants (Drorbaugh and Fenn, 1955) and was subsequently adapted for rodents being used in long-term studies where repeated measurements of lung function were required (Pennock et al., 1979).

By placing the animal in a transparent plexiglas chamber where it is unrestrained, the box pressure signal generated by the respiratory movements allows the analysis of breathing pattern. Although this technique has been severely criticised and cannot be considered as an equivalent to ventilatory mechanics (Adler et al., 2004; Bates et al., 2004), it presents the major advantage that anaesthesia and tracheal intubation are not required and that an estimator of airflow limitation, called enhanced pause (Penh), might be calculated. In cats, BWBP can be performed without sedation, which might sometimes be necessary in dogs which are panting (Rozanski and Hoffman, 2004; Talavera et al., 2005).

Chronic airway diseases, such as asthma and chronic bronchitis, are important pathologies in feline respiratory medicine (Bay and Johnson, 2004). Increased AR using ventilatory mechanics has been described in clinical cases of asthma and bronchitis (Dye et al., 1996) as well as in experimental models of feline asthma (Norris et al., 2003; Padrid et al., 1995). Hoffman et al. (1999)

showed that AR tests are feasible using BWBP in healthy adult cats. Furthermore, AR has been shown to be lower in 12-year-old healthy cats than in 2-year-old healthy cats, i.e., the concentration of agonist inducing bronchoconstriction was higher in older than in younger cats (Hirt et al., 2003). However, the repeatability of AR tests in cats, their predictive value with regard to airway inflammation and protective effect of bronchodilator treatment have not yet been investigated.

The objectives of this study were (1) to test repeatability of AR tests using BWBP in healthy cats, (2) to compare two protocols of AR tests using different concentrations of a same bronchoconstrictive agent, (3) to assess the effect of ageing on AR in healthy cats over a period of 2 years, (4) to assess the effect of airway inflammation on AR in an experimental model of feline asthma and (5) to test whether a administration of a bronchodilator modifies AR in healthy cats.

2. Materials and methods

2.1. Animals

Thirty shorthair cats (Harlan Netherlands: 16 neutered males, 14 intact females) 10 months to 3 years of age, with body scores 2.5–4 (min: 1, max: 6; Scarlett et al., 1994), and body weights 2.9–5.5 kg were used in the study. The animals were housed and cared for according to the national guidelines and the principles advised by the European Council for the Care of Laboratory Animals. They were housed in groups in appropriate rooms where they were allowed to move freely and received *ad libitum* water. They received twice-daily canned and dry food (~ 60 kcal/kg bodyweight/day). A light:dark cycle of 12:12 h was maintained. Female cats displayed only mild signs of oestrus when they reached the age of 8–10 months. Room temperature and humidity were recorded daily and ranged between 20 and 23 °C and 35 and 50%, respectively. Each cat was weighed weekly. The animals underwent regular routine clinical examination and lung auscultation. The study was approved by the Animal Ethical Committee of the University of Liège.

2.2. Study design

2.2.1. Repeatability of airway responsiveness testing

All cats ($n = 30$) underwent AR testing on two occasions (Test 1 and Test 2). Both tests were performed following the same procedure as described in the following section. The mean interval between both tests was 1.1 ± 0.5 months and the mean age of the animals was 15 ± 7 months. At the time of testing, all animals were healthy; allergen sensitisation was performed in some cats at a later time point (see below).

2.2.2. Comparison between slowly and rapidly increasing carbachol concentrations

In order to evaluate if the calculated AR was influenced by the nebulization protocol used, ten healthy cats underwent, in a random order, at a 2-week interval, two AR tests, during which the stepwise increase of carbachol concentrations was based on either “large fractions” (0.005%, 0.01%, 0.02%, 0.04%, 0.06% and 0.08% carbachol diluted in saline) or “small fractions” (0.005%, 0.01%, 0.015%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07% and 0.08%).

2.2.3. Evolution of airway responsiveness in healthy cats aged 12–36 months

In 15 healthy control cats, repeated AR tests performed between 12 and 36 months of age were available. In order to follow AR during this time period, AR tests performed when the cats aged approximately 12, 18, 24, 30 and 36 months were considered and compared to values obtained at 12 months.

2.2.4. Impact of placebo and allergen exposure on airway responsiveness in healthy and allergen-sensitised cats

Twelve healthy control cats and 12 cats previously sensitised with *A. suum* underwent AR testing before and after a placebo or allergen exposure. Furthermore, chest radiography, bronchoscopy and BAL were performed. Pre-challenge tests were undertaken one month before placebo or allergen challenge. The impact of allergen-induced airway inflammation on AR, radiography and bronchoscopy scores was evaluated and correlation analysis between variables was performed.

2.2.5. Impact of bronchodilator treatment on airway responsiveness

In order to test whether a preventive administration of a short-acting bronchodilator would decrease AR, four healthy cats inhaled 1 h before undergoing AR test either a saline or a salbutamol solution. After a wash-out period of 3–5 days, treatments were inverted and AR tests were repeated.

2.3. Barometric whole body plethysmography (BWBP)

The system for BWBP for cats described elsewhere in detail was used (Kirschvink et al., 2005a,b,d). Briefly, the cats were placed in the transparent plexiglas chamber of a barometric whole body plethysmograph (Buxco Electronics; height: 25 cm, length: 51 cm, width: 30 cm) that was ventilated by continuous bias flow (Air Control Industries Ltd.) at 4 L/min, which was adjustable by a flowmeter (Influx, Caché Instrumentation Ltd.). Air inlet via a screen pneumotachograph (35 mm diameter) and air outlet were at the opposite ends of the chamber.

A differential pressure transducer (Emka Technologies) sampled pressure signals at 100 Hz by use of the IOX software version 1.569 (Emka Technologies), thereby providing a breath-by-breath analysis of waveforms.

The following respiratory variables were recorded: respiratory rate (RR [cycles/min]), inspiratory time (T_i [ms]), expiratory time (T_e [ms]), relaxation time (R_T [ms], i.e. the time point when 65% of tidal volume is expired), peak inspiratory and expiratory pseudo-flows (PIF and PEF [mL/s]), estimated tidal volume (V_T [mL]), estimated minute ventilation (V_E [mL/min]). The enhanced pause, an unitless index of air-flow limitation being correlated with pulmonary resistance (Halloy et al., 2004; Hamelmann et al., 1997; Kirschvink et al., 2005c), was calculated as follows: $Penh = ([T_e - R_T]/R_T \times PEF/PIF)$. Waveforms disturbed by movements and sniffing were automatically eliminated. The chamber pressure signal was calibrated before each use and monitoring of temperature and relative humidity of the room where BWBP was performed daily.

2.3.1. Determination of airway responsiveness

After a 5 min baseline record period of respiratory variables, an ultrasonic nebuliser (Ultraneb Devilbiss 2000, Devilbiss Healthcare Inc.) was connected to the nebuliser port of the plethysmograph and sterile saline (0.9% NaCl) was nebulised for 1 min. A post-saline record of 5 min was performed and saline solutions containing increasing concentrations of carbachol (Sigma) were successively nebulised for 1 min and were each followed by 5 min recording until bronchoconstriction occurred. Bronchoconstriction was defined as an increase of 300% or more of post-saline $Penh$ lasting 20 s or longer. The carbachol concentration inducing a 300% increase of post-saline $Penh$ (C- $Penh300$) was calculated by interpolation of the dose–response curve between the two final concentrations used (Hirt et al., 2003; Hoffman et al., 1999). “Large fractions” of carbachol were only used when they were compared with “small fractions”.

2.4. *Ascaris suum* sensitisation

A. suum antigen (Greer Laboratories Inc.) sensitisation was achieved by two intramuscular (IM) injections of a freshly prepared adjuvant-allergen emulsion (TiterMax, Alexis Corporation) that were administered two weeks apart (Kirschvink et al., 2005a; Padrid et al., 1995). The cats were examined daily for four days after each injection and no adverse reactions were observed. Two to four weeks after the second injection, the AS-group underwent a single 5 min challenge with an aerosol containing 0.01% of AS. The control cats underwent a 5-min placebo challenge by inhaling sterile saline (NaCl 0.9%).

2.5. Chest radiography

All cats underwent chest radiography 24 h after placebo or allergen challenge and 24 h prior to bronchoscopy. Ventro-dorsal and right lateral view pictures were made without sedating the cats. A scoring system described by Foster et al. (2004) that ranged for the total score from 0 to 6 was used to evaluate separately the bronchial pattern and the interstitial pattern. The following keys were applied for the scoring of the bronchial pattern: 0: absence of signs, 1: mild (primary bronchi visible), 2: moderate (secondary bronchi visible), 3: severe (tertiary bronchi visible). Interstitial pattern was scored as follows: 0: absence of signs, 1: mild (mild interstitial framework visible), 2: moderate (interstitial framework distinguishable from a bronchial pattern), 3: severe (clearly apparent interstitial pattern). This scoring system was applied to each pair of views by a radiologist (FS) blinded to the animals' identity and history.

2.6. Bronchoscopy and bronchoalveolar lavage

Bronchoscopy was performed 72 h after placebo or allergen exposure. The cats were sedated by IM injection of medetomidine (100 µg/kg; Domitor, Pharmacia Animal Health). The cats were fitted with an antebrachial intravenous (IV) catheter and anaesthesia was induced and maintained by IV administration of propofol (1–2 mg/kg; Diprivan, Zeneca). After topical anaesthesia of the larynx (lidocaine; Linisol 1%, Braun Medical), a paediatric 4.8 mm diameter video-endoscope (Fujinon EB-4105, ONYS) was introduced through the oral cavity and the trachea into the right main bronchus, allowing a macroscopic evaluation of the trachea and the large airways. A bronchoscopy score taking into account the mucosal aspect (0: normal, 1: moderate mucosal reddening and oedematous appearance, 2: severe reddening and oedematous appearance), the presence of mucus (0: absent, 1: moderate, 2: abundant) and the airway reactivity (i.e., coughing, tracheal and bronchial mobility or collapse: 0: absent, 1: moderate, 2: severe) was established. When the bronchoscope was wedged, 10 mL of preheated (37 °C) sterile saline (NaCl 0.9%) was instilled into the right main bronchus and recovered by gentle suction. The same procedure was repeated in the left main bronchus. Recovery of bronchoalveolar lavage fluid (BALF) ranged from 50% to 70%. After extraction of the bronchoscope, the cats were oxygenated for 3 min by breathing spontaneously oxygen-enriched air. Anaesthesia was reversed by IV administration of atipamezole (250 µg/kg; Antisedan, Pharmacia Animal Health).

The recovered BALF was kept on ice until being processed within 1 h of collection. Cytology and total nucleated cell counts were performed both by light

microscopy after Cytospin (Cytospin 2, Shandon Inc.) centrifugation followed by Giemsa staining and after Türk coloration and use of a Thomas cell, respectively. A semi-quantitative bacteriological analysis of BALF was systematically performed and was negative in all cases ($<1.7 \times 10^3$ colonies/mL) (Peeters et al., 2000).

2.7. Bronchodilator treatment

The cats were placed for 1 min into the plethysmograph and were nebulised in a random order once with sterile saline (NaCl 0.9%) and once with salbutamol (1 mg/mL in NaCl 0.9%; Ventolin, GSK). Airway reactivity was determined 1 h after treatment. After a wash-out period ranging between 3 and 5 days, treatments were inverted and AR tests were repeated.

2.8. Statistical analysis

All variables were normally distributed and are shown as mean $\pm$ standard deviation (SD). Data were analysed by analysis of variance (ANOVA) for repeated measures for assessment of the effect of age on AR. Effect of placebo and AS-exposure in control and AS-cats was assessed by use of ANOVA-2, whereas paired comparisons were performed using Student's *t* tests (Statview, version 5, SAS Institute Inc.). Correlation analyses were performed and illustrated by use of linear regression. A *P*-value lower than 0.05 was considered to be significant.

3. Results

Assessment of AR by use of BWBP as a method of lung function monitoring and the use of carbachol as the bronchoconstrictive agent was well tolerated by all cats. Indeed, the cats were lying quietly in the plethysmograph until onset of the response to the carbachol concentration inducing airflow limitation. Bronchoconstriction occurred within 1–5 min after the inhalation challenge and was preceded by increased or changing RR, movements and mild cholinergic signs, such as swallowing saliva. The cats were either lying or remained seated when bronchoconstriction was clinically visible by an expiratory dyspnoea. Coughing was observed in some cats. Peak response, measured as the maximal value of Penh, was always recorded within 10 min after the last challenge and completely resolved within 15 min. Use of bronchodilators or oxygen supply were never necessary.

3.1. Repeatability of airway responsiveness testing

As shown by a scatter plot in Fig. 1, the concentrations inducing a 300% increase of Penh were very similar

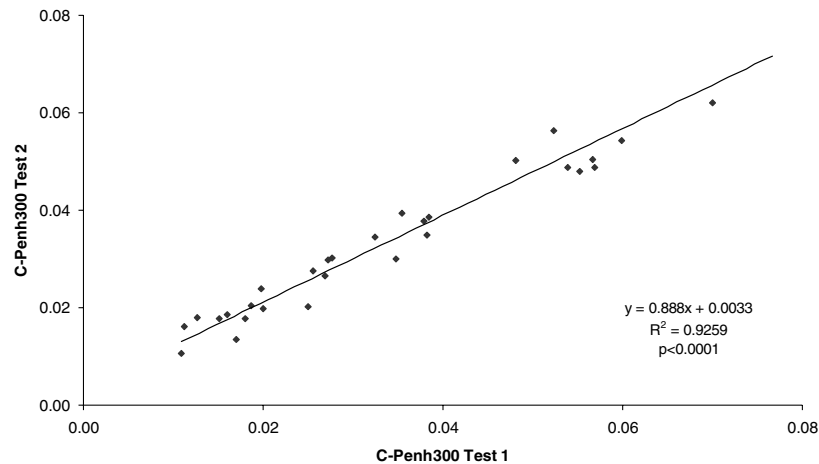


Fig. 1. Scatter plot of C-Penh300 (%) determined at Test 1 and Test 2 ($n = 30$). Equation of linear regression, coefficient of determination and P -value are indicated.

and significantly correlated between Test 1 (mean C-Penh300 Test 1: $0.0345 \pm 0.0177\%$) and Test 2 (mean C-Penh300 Test2: $0.0339 \pm 0.0163\%$). C-Penh300 values obtained in Test 1 ranged from 0.0109% to -0.0766% and those in Test 2 ranged from 0.0107% to 0.0794%. Mean variation of C-Penh300 was $0.0039 \pm 0.0028\%$, which corresponded to a relative change from Test 1 of $12.6 \pm 9.5\%$ (minimum 0.5%, maximum 37%). A coefficient of variation (CV) was calculated for each cat result. Mean CV was $8.9 \pm 6.8\%$ (min: 0.5%, max: 25%).

3.2. Comparison between slowly and rapidly increasing carbachol concentrations

Despite a large inter-individual variability the mean C-Penh300 value calculated using “small fractions” (C-Penh300: $0.0368 \pm 0.0127\%$) was significantly lower than that obtained using “large fractions” (C-Penh300: $0.0393 \pm 0.0149\%$, $P < 0.05$) of carbachol. The mean decrease of carbachol concentration was $0.0028 \pm 0.0027\%$ (minimum: 0.0001%, maximum: 0.0047%) and the mean relative decrease was $5.8 \pm 5.7\%$ (minimum: 0.2%, maximum 13.6%). A scatter plot shown in Fig. 2 illustrates that both results were significantly correlated.

3.3. Evolution of airway responsiveness in healthy cats aged 12–36 months

In comparison to AR-values determined at the age of 12 months, C-Penh300 was significantly increased at 18, 24 and 30 months (Fig. 3). As data from only four cats were available at the age of 36 months, difference from baseline did not reach significance ($P = 0.07$).

3.4. Impact of placebo and allergen exposure on airway responsiveness in healthy and allergen-sensitised cats

Bronchial responsiveness was similar at pre-challenge in both cat groups and remained unchanged after saline challenge in control cats, whereas a significant decrease of C-Penh300 was recorded in AS-sensitised cats (Fig. 4a). However, C-Penh300 of AS-cats at post-challenge did not significantly differ from that recorded in controls. The radiography and bronchoscopy scores of control cats did not differ between pre- and post-challenge and were similar to that of AS-cats at pre-challenge. Allergen exposure induced a significant increase of the radiography and bronchoscopy scores in AS-sensitised cats, which were also significantly different from scores recorded in controls (Figs. 4b and c). Cytological analysis of BALF is shown in Table 1. Whilst saline challenge did not induce significant changes of total and differential cell counts in control cats, allergen challenge significantly increased eosinophil count and eosinophil percentage in AS-cats. Neutrophil count and percentage did not increase in a significant manner. A negative correlation between BALF eosinophil percentage, BALF granulocyte percentage (neutrophils and eosinophils) and C-Penh300 was found (respectively, $r = -0.34$, $P < 0.05$ and $r = -0.36$, $P < 0.01$).

3.5. Impact of bronchodilator treatment on airway responsiveness

C-Penh300 calculated after saline nebulization was similar to that obtained in other healthy cats. Preventive salbutamol nebulization did not affect baseline Penh-measurements 1 h after administration (data not shown), but significantly increased C-Penh300 (Fig. 5).

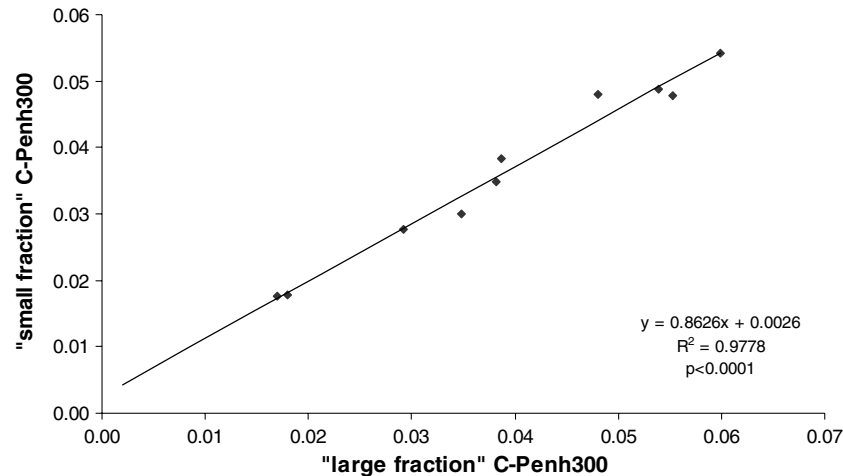


Fig. 2. Scatter plot of C-Penh300 (%) determined with "small fractions" and "large fractions" of carbachol ($n = 10$). Equation of linear regression, coefficient of determination and P -value are indicated.

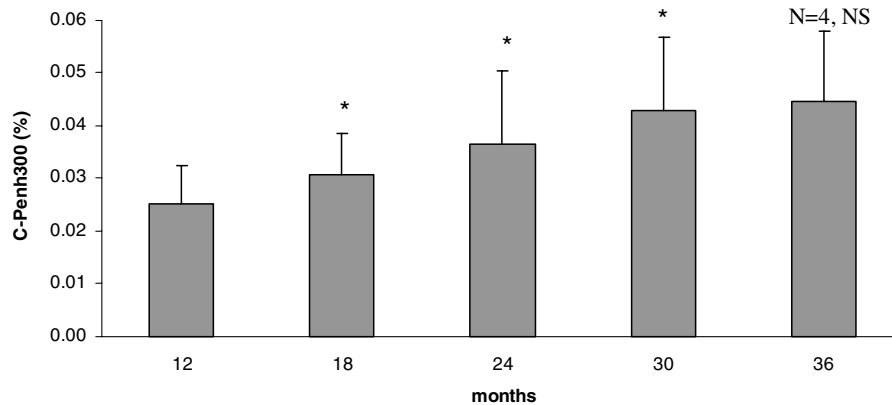


Fig. 3. Carbachol concentration inducing a 300% increase of Penh (C-Penh300) determined in 15 healthy cats at 12, 18, 24, 30 and 36 months of age. At 36 months, data from only four cats are available. Data are shown as mean $\pm$ SD. *Significantly different from 12 months, $P < 0.05$.

In two cats, the highest carbachol concentration tested (0.1%) did not induce bronchoconstriction and C-Penh300 was arbitrarily set at 0.11% for statistical analysis.

4. Discussion

This study aimed at determining whether AR tests performed by use of BWBP were repeatable in cats and to what extent bronchial reactivity was affected by the nebulization protocol used, the animals' age, the airways' inflammatory status and a bronchodilator treatment.

By performing the same AR test in 30 cats at approximately a one month interval, repeatability was evaluated. Inter-individual variability was observed in these healthy cats, but C-Penh300 values obtained in each cat were very similar between Test 1 and Test 2. Although it would have been preferable to repeat the

test on three occasions or more, these results suggest that assessment of AR by applying the protocol as described was repeatable. The larger incremental steps during carbachol administration (large fractions compared to small fractions) significantly increased C-Penh300 because the lowest concentration inducing bronchoconstriction was more likely to be detected using small incremental increases. This result shows to what extent modifications of the nebulization protocol might affect the AR-result.

Accordingly, the high C-Penh300 values reported in earlier studies (Hirt et al., 2003; Hoffman et al., 1999) are probably due to methodological aspects rather than to differences between the cats' health status. Differences between C-Penh300 have also been found in healthy dogs when different types of nebulisers (jet compared to ultrasound nebuliser) and different ventilation rates of the bias flow were used (Talavera et al., 2005b). From a practical point of view, the use of rapidly or slowly increasing concentrations of carbachol has probably

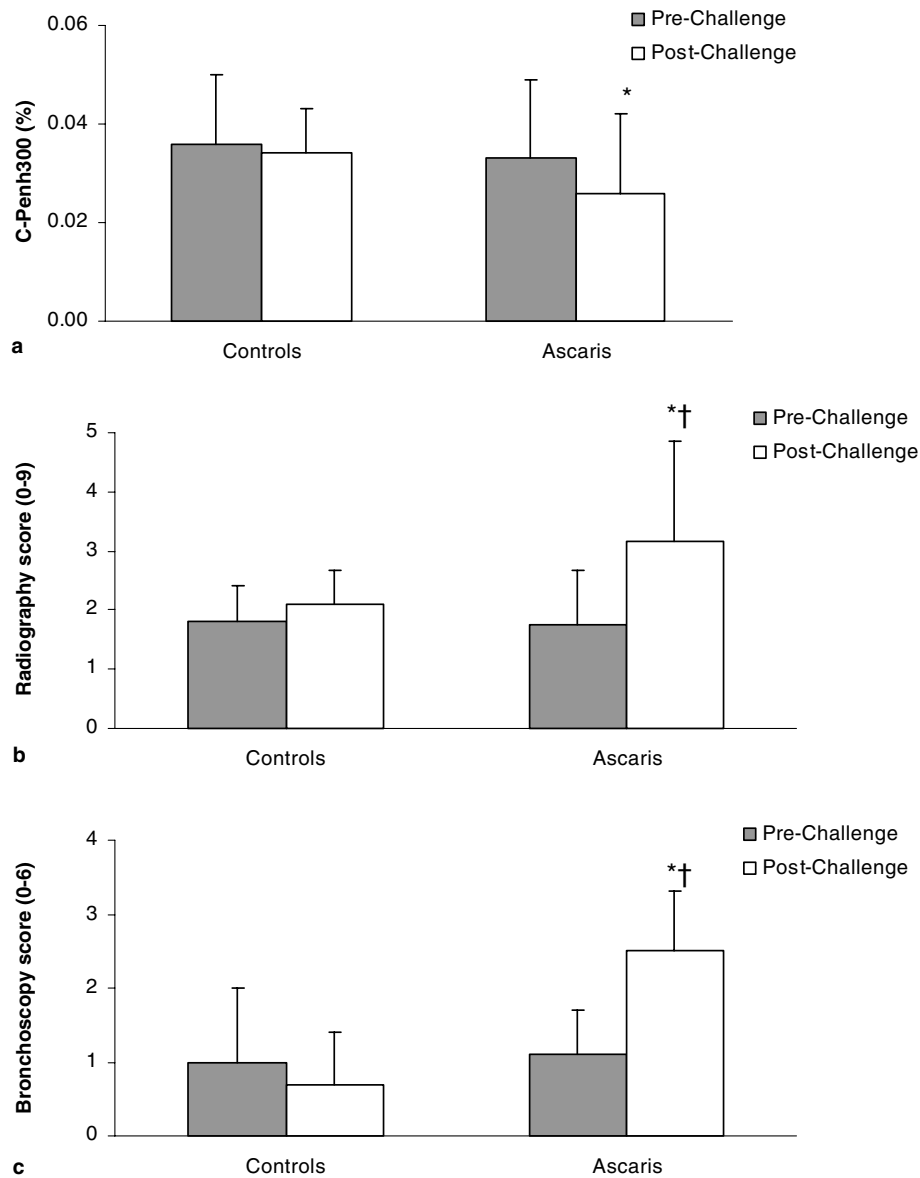


Fig. 4. Carbachol concentration inducing a 300% increase of Penh (C-Penh300) (a), radiography score (b) and bronchoscopy score (c) determined in 12 healthy control cats and 12 *A. suum*-sensitised cats before (pre-challenge) and after saline or allergen challenge. Data are shown as mean $\pm$ SD. *Significantly different from respective Pre-challenge value, †Significantly different from respective control-value, $P < 0.05$.

no effect on the result, given that the change of C-Penh300 is very small for each individual. However, the use of “small fractions” increases the time necessary for performing the AR test. On the other hand, although this observation was not shown here, the use of “large fraction” concentrations induced bronchoconstriction of a higher intensity and longer duration and should not be recommended. These results suggest that a standardised procedure for AR testing should be defined if comparisons of results of different research units are to be made.

An age-related decrease of AR has been demonstrated in studies in healthy humans (Burney et al., 1987; Davis and Byard, 1988) as well as in clinically healthy cats (Hirt

et al., 2003). The latter study compared young adult cats (approximately 2 years old) with old adult cats (approximately 12 years old) and described a tenfold increase of C-Penh300 for the older cat group. The AR tests were repeated in some young and old adult cats one year later and no significant increase of C-Penh300 was reported. During the investigation period of 15 healthy cats in our study, a significant increase of C-Penh300 was recorded between the ages of 12 and 30 months, suggesting that AR continuously increases in young adult cats. These different results may be due either to the small number of young cats that were investigated twice in the study of Hirt et al. (2003) or to differences between nebulisers, carbachol concentration steps, hardware

Table 1

Cytology of bronchoalveolar lavage fluid collected in healthy control cats and *A. suum*-sensitised cats before (PRE) and after (POST) saline or allergen exposure

Variable	Time point of sampling	Control cats (<i>n</i> = 12)	AS-sensitised cats (<i>n</i> = 12)
Total cells ($\times 10^3$ cells/mL)	PRE	226 $\pm$ 233	228 $\pm$ 181
	POST	216 $\pm$ 249	219 $\pm$ 121
PMN ($\times 10^3$ cells/mL)	PRE	26.2 $\pm$ 38.9	22.6 $\pm$ 17.8
	POST	21.7 $\pm$ 29.6	36.0 $\pm$ 31.6
Eosinophil ($\times 10^3$ cells/mL)	PRE	3.2 $\pm$ 3.3	5.2 $\pm$ 3.2
	POST	9.6 $\pm$ 6.1	79.1 $\pm$ 23.8*
% PMN	PRE	10 $\pm$ 4	12 $\pm$ 3
	POST	10 $\pm$ 3	20 $\pm$ 14
% Eosinophil	PRE	3 $\pm$ 3	3 $\pm$ 2
	POST	4 $\pm$ 4	42 $\pm$ 19*

Data are shown as mean $\pm$ SD.

AS: *A. suum*.

PMN: polymorphonuclear cells.

* Significantly different from respective PRE-value and from controls, $P < 0.05$.

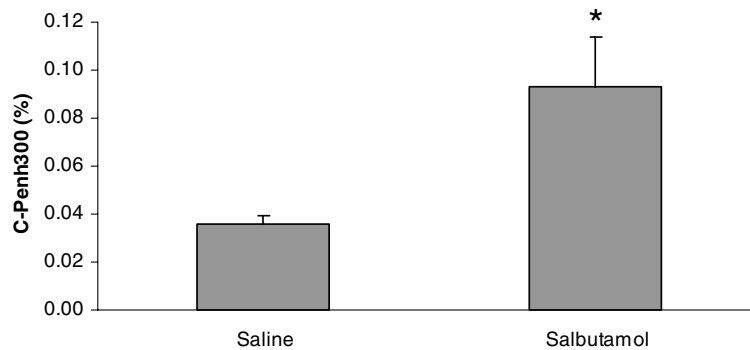


Fig. 5. Carbachol concentration inducing a 300% increase of Penh (C-Penh300) in healthy cats (*n* = 4) determined 1 h after saline or salbutamol inhalation. Data are shown as mean $\pm$ SD. *Significantly different from saline-value, $P < 0.05$.

and software used. Furthermore, breed- and body size-related differences might also influence the results. As many clinical cases of asthma or bronchopulmonary diseases are diagnosed in young and middle-aged cats (Bay and Johnson, 2004; Dye et al., 1996), further investigations allowing completion of the present data should be performed in healthy cats that are older than three years.

Allergen-induced eosinophilic and neutrophilic airway inflammation in AS-sensitised cats was associated with radiographic and bronchoscopic changes observed in feline asthma (Bay and Johnson, 2004). Cytological findings at pre-challenge and in healthy control cats were similar to reference values (Hawkins, 2004) and changes of BALF cytology after allergen exposure was in agreement with previous studies also describing experimental models of feline asthma (Norris et al., 2003; Padrid et al., 1995). Regarding AR, a significant decrease of C-Penh300 at post-challenge was observed in the AS-sensitised group. However, the difference from control cats was not significant, which was not the case in other experimental models (Norris et al.,

2003; Padrid et al., 1995) or in clinical cases of asthma (Dye et al., 1996). This may be because the cats used in our study were investigated after a single rather than after repeated allergen exposure. The inflammatory process is likely to be more pronounced and airway remodelling might contribute to bronchial hyper-reactivity if repeated allergen exposure occurs (Bousquet et al., 2000). On the other hand, all previous studies investigated lung function by measuring ventilatory mechanics under general anaesthesia, which might be more sensitive to moderate changes of airflow limitation than BWBP. Nevertheless, the significant negative correlation found between C-Penh300 and BALF eosinophil and granulocyte percentage suggested that AR can be used as a non-invasive indicator of lower airway inflammation.

To test whether AR would be reduced if bronchial narrowing was prevented by administering a bronchodilator, a single dose of salbutamol was administered by nebulization before AR was measured in healthy cats. Salbutamol had no effect on baseline Penh (data

not shown), but significantly increased C-Penh300 in comparison to the placebo. This result indicates that broncho-protection, reflected by a decrease of AR, occurred in response to a bronchodilator treatment. Similar results have been reported in piglets that were treated with clenbuterol before undergoing an inhaled acetylcholine challenge (Halloy et al., 2004) and in salbutamol pre-treated mice (Hamelmann et al., 1997).

Other studies using BWBP in rodents and cats have demonstrated that anti-inflammatory agents also reduce AR and suggest that this non-invasive assessment of AR might be suitable to monitor response to treatment (Kirschvink et al., 2005d; Kumar et al., 2003; Toward et al., 2005). Although our results need to be confirmed in future studies by different types of bronchodilators or anti-inflammatory agents and by including a larger number of cats, they strongly suggest that BWBP might be used to monitor AR in response to treatment.

In this study, the only bronchoconstrictor used was carbachol, a parasympathomimetic agent. Carbachol has been successfully used in earlier studies investigating AR in cats (Hirt et al., 2003; Hoffman et al., 1999) and bears the advantage of inducing bronchoconstriction of sufficient duration to be assessed by BWBP. An alternative to this pharmacological agent would have been methacholine or acetylcholine, which have both been used under anaesthesia in cats (Dye et al., 1996; Padrid et al., 1995). Other bronchoconstrictive mediators, such as histamine, which specifically acts on H₁-receptors, hypertonic saline or adenosine 5'-monophosphate (AMP), considered as non-specific mediators, might also be of interest (O'Byrne, 1998). However, histamine induces concentration-dependent contraction or dilatation of isolated tracheal and bronchial smooth muscle in cats (Blaber and Fryer, 1985). In healthy cats, inhalation of histamine has been reported to induce variable responses and therefore appears as an inappropriate bronchoconstrictor agent for this species (Blaber and Fryer, 1985; Dye et al., 1996). Preliminary results suggest that AMP does not induce bronchoconstriction in healthy cats and its use for assessment of AR in cats with lower airway inflammation remains to be investigated (Hirt and Vondrakova, 2005).

5. Conclusion

The present study provides evidence that BWBP could be of value for monitoring AR in cats. The AR test such as described here was well tolerated by the animals and was repeatable. Our data also show that the inter-individual variability of AR in healthy cats was considerable, which implies that AR test did not allow discrimination between healthy and allergen-sensitised asthmatic cats. Accordingly, AR should at this time be

considered in combination with other inflammatory indicators, such as BALF cytology, thoracic radiography or bronchoscopy. If the same cat is compared to itself, the assessment of AR appears to be promising as a non-invasive indicator of lower airway inflammation or for monitoring response to bronchodilator treatment.

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