Repetitive hyperpnoea causes peripheral airway obstruction and eosinophilia

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Repetitive hyperpnoea causes peripheral airway obstruction and eosinophilia. M.S. Davis, A.N. Freed. ©ERS Journal Ltd 1999.

ABSTRACT: Hyperpnoea of canine peripheral airways with dry air results in airway obstruction, mucosal damage, and inflammation. The purpose of this study was to evaluate the effect of repeated dry air challenge (DAC) on airway obstruction, reactivity and the development of airway inflammation in dogs.

Canine peripheral airways received DAC (delivered under general anaesthesia through a bronchoscope) every 48 h for two weeks. Peripheral airway resistance and reactivity were measured prior to each DAC. After the final DAC, bronchoalveolar lavage fluid (BALF) cells and soluble mediators from challenged and control airways were measured.

Repeated bronchoscopy had no effect on airway mechanics. Repeated DAC produced cumulative increases in peripheral airway resistance and peak obstructive response to DAC. The response to hypocapnia was also increased in airways receiving repeated DAC. However, when the response to agonists was expressed as a change from baseline, consistent significant increases were not observed. Repeated bronchoscopy produced insignificant changes in BALF cells and eicosanoid mediators. Repeated DAC produced marked eosinophilic inflammation and increased prostaglandins D₂, E₂, and F_{2α}, as well as leukotrienes C₄–E₄.

In conclusion, repeated dry air challenge in dogs *in vivo* causes persistent airway obstruction and inflammation not unlike that found in human asthma.

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Epidemiological studies reveal an increased incidence of asthma in winter sport athletes who routinely experience hyperpnoea during training under frigid conditions [1–5]. In one study, ~80% of the athletes exhibited asthma or asthma-like symptoms, compared with only 3% of a reference population [2]. These symptoms persisted throughout the warmer months in these individuals. The authors of this study have suggested that repeated episodes of hyperpnoea with cold air may predispose subjects to the development of asthma.

The acute effects of hyperpnoea on airway function have been well documented. Hyperpnoea with frigid air is known to cause peripheral airway cooling in both normal and asthmatic subjects [6–8]. In asthmatic subjects, transient airway obstruction occurs after hyperpnoea (hyperpnoea-induced bronchoconstriction; HIB) [6]. A canine model of HIB has been developed to investigate mechanisms that may be involved in exercise-induced asthma [9]. In this model, room temperature dry air is delivered at high flow rates through a bronchoscope into a wedged sublobar segment (dry air challenge; DAC). This manipulation produces the same thermal profile (presumably due to the same degree of evaporative cooling) as that produced at the same segmental level in humans hyperventilating with frigid air [7, 10]. Similarities between hyperventilation with frigid air in humans and DAC in the canine model extend to the timing of both the early [9] and late phase obstruction [11, 12], as well as the cellular

and lipid mediator changes observed in the post-challenge bronchoalveolar lavage fluid (BALF) [11, 13, 14].

Based on the epidemiological studies in winter sport athletes [1–5], it was hypothesized that repeated hyperpnoea with dry air causes chronic airway inflammation and airway obstruction. To test this hypothesis, the cumulative effects of repeated DAC on peripheral airway mechanics and inflammation were examined in a canine model of cold air hyperpnoea.

Material and methods

Study design

In each dog, three airways in separate lobes were wedged with a bronchoscope on the first day of the protocol. The location of the wedged bronchoscope was recorded in each airway by constructing a map of the proximal airway branches leading to the wedge site. Using this map, the bronchoscope could be advanced on subsequent days to the same wedge site. Two mapped airways in each dog were designated repeated challenge airways (repeated DAC). In one airway, peripheral airway resistance (R_{pa}) and maximal peripheral airway resistance to hypocapnia (R_{pa,max,CO_2}) were measured prior to DAC (n=6). In the second airway, only R_{pa} was measured prior to DAC (n=5). The third mapped airway served as the control for the repeated wedging of the bronchoscope (repeated wedge), and only R_{pa} and R_{pa,max,CO_2} were

measured ($n=6$). DAC was not performed in this airway. The experimental protocol was repeated on the same airways every 48 h for 2 weeks, for a total of eight DACs per challenged airway. On the final day, the three principal airways and a fourth airway were lavaged. BALF from the fourth airway (control) represented a baseline for the effects of repeated anaesthesia, and served as a control for the effect of repeated bronchoscopy.

Because a single DAC is known to cause changes in BALF cells and eicosanoids, the BALF from repeated DAC airways was compared with BALF obtained 10 min after a single DAC in a naive airway (single DAC) ($n=10$). Recovered BALF was analysed in a manner identical to that used for the BALF recovered from other airways. Results from the analysis of BALF from single DAC airways were compared to results from repeated DAC airways to differentiate between changes caused by the most recent DAC and those present as a result of the repeated DAC.

Methods

All animals were handled and maintained in accordance with the Policy and Procedures Manual published by the Johns Hopkins University School of Hygiene and Public Health's Animal Care and Use Committee (ACUC). The ACUC is sanctioned by the American Association of Accreditation for Laboratory Animal Care.

Measurement of peripheral airway resistance. Male mongrel dogs were anaesthetized with an intravenous bolus of thiopental ($25 \text{ mg} \cdot \text{kg bodyweight}^{-1}$) and fentanyl ($1 \text{ } \mu\text{g} \cdot \text{kg bodyweight}^{-1}$), and maintained under anaesthesia with additional intravenous boluses of fentanyl ($1 \text{ } \mu\text{g} \cdot \text{kg bodyweight}^{-1}$) every 15 min. The depth of anaesthesia was assessed by heart rate, blood pressure, canthal reflex, and the presence of spontaneous movements and breathing. Dogs were intubated and mechanically ventilated ($17 \text{ mL} \cdot \text{kg bodyweight}^{-1}$) with room air. End-tidal CO_2 was monitored with a CO_2 analyser (Beckman LB-2; Beckman Inc., Anaheim, CA, USA) and maintained at $\sim 4.5\%$ by adjusting ventilator frequency. Heart rate and blood pressure were monitored via a noninvasive monitor (Data-scope Accutorr 1A; Datascope Corp., Paramus, NJ, USA), and rectal temperature was monitored using a telethermometer (Yellow Springs Instrument Co., Yellow Spring, OH, USA). A bronchoscope (Olympus BF Type P10; Olympus Corp. of America, New Hyde Park, NY, USA, 5 mm o.d.) was inserted through an airtight portal of the endotracheal tube and gently wedged into a sublobar segmental bronchus. The suction port (1.2 mm) of the bronchoscope was connected to a pressure transducer (Statham, Gould Inc., Oxnard, CA, USA) which was used to measure airway pressure in the subtended lung segment (P_{sls}). Compressed, dry, room temperature, $5\% \text{ CO}_2$ in air was delivered at a rate of $200 \text{ mL} \cdot \text{min}^{-1}$ through the suction port of the bronchoscope into the wedged sublobar segment. R_{pa} was measured by stopping the ventilator during exhalation, such that the unobstructed areas of the lung equilibrated with atmospheric pressure at functional residual capacity. Under these conditions, P_{sls} decays to a plateau at a pressure greater than the alveolar pressure (atmospheric) in the surrounding unobstructed lung so that $R_{\text{pa}} = P_{\text{sls}}/200 \text{ mL} \cdot \text{min}^{-1}$.

Airway reactivity to hypocapnia. To assess nonspecific airway reactivity, insufflation of dry $5\% \text{ CO}_2$ in air was switched to $0\% \text{ CO}_2$ at $200 \text{ mL} \cdot \text{min}^{-1}$ for 3 min. Reactivity was recorded as the peak R_{pa} at the end of the 3 min challenge ($R_{\text{pa,max,CO}_2}$) and the peak change from baseline R_{pa} ($\Delta R_{\text{pa,max,CO}_2}$).

Dry air challenge. DAC was performed by increasing the insufflation of dry $5\% \text{ CO}_2$ in air from 200 to $2,000 \text{ mL} \cdot \text{min}^{-1}$ for 5 min. Airflow was then returned to $200 \text{ mL} \cdot \text{min}^{-1}$ and R_{pa} was monitored over the next 15 min. Reactivity to DAC was recorded as the peak R_{pa} recorded after the challenge ($R_{\text{pa,max,DAC}}$) and the peak change from baseline R_{pa} ($\Delta R_{\text{pa,max,DAC}}$).

Bronchoalveolar lavage fluid recovery and analysis. Three 20-mL aliquots of warmed Hanks' buffered saline were infused through the bronchoscope into the wedged sublobar segment, and then recovered by gentle aspiration. The recovered fluid was pooled and the volume determined. Nucleated cells were counted using a haemocytometer, and cytocentrifuged cells stained with a modified Wright-Giemsa stain were used for differential counts of nucleated cells. The remainder of the sample was centrifuged, and the supernatant was passed through a C-18 Sep-Pak exchange cartridge (Waters Corp., Milford, MA, USA) and eluted with 4 mL methanol. Aliquots of the eluted sample were dried under vacuum, reconstituted with buffered saline, and analysed using commercially available enzyme-linked immunosorbent assay (ELISA) kits for thromboxane ($\text{Tx} \text{B}_2$), prostaglandin ($\text{PG} \text{E}_2$, $\text{PGF}_{2\alpha}$ (Neogen Inc., Lexington, KY, USA), and PGD_2 (Cayman Chemical Inc., Ann Arbor, MI, USA), and sulphidopeptide leukotrienes $\text{C}_4\text{--E}_4$ (Neogen Inc.).

Analysis

R_{pa} , $R_{\text{pa,max,CO}_2}$ and $R_{\text{pa,max,DAC}}$ data were analysed using the Friedman repeated measures analysis of variance (ANOVA). The Student-Newman-Keuls test applied to ranks was used to compare individual treatment means. The Mann-Whitney rank sum test was used to compare BALF cellular and biochemical data. Statistical significance in all cases was judged at $p < 0.05$. All values are expressed as the mean $\pm$ SEM.

Results

Baseline R_{pa}

Repeated DAC of canine peripheral airways increased baseline R_{pa} , whereas repeated wedging did not significantly alter baseline R_{pa} (fig. 1). Baseline R_{pa} on day 1 was $0.80 \pm 0.13 \text{ cmH}_2\text{O} \cdot \text{mL}^{-1} \cdot \text{s}$ in the repeated wedge airways ($n=6$), which was similar to that in the repeated DAC airways on day 1 ($0.80 \pm 0.079 \text{ cmH}_2\text{O} \cdot \text{mL}^{-1} \cdot \text{s}$, 11 airways in six dogs). Baseline R_{pa} of repeated wedge airways did not significantly change throughout the duration of the experiment ($p=0.659$). However, in repeated DAC airways base-line R_{pa} increased with repeated challenge (fig. 1; $p=0.033$). Baseline R_{pa} was significantly higher on days 9 ($1.24 \pm 0.19 \text{ cmH}_2\text{O} \cdot \text{mL}^{-1} \cdot \text{s}$), 11 ($1.29 \pm 0.25 \text{ cmH}_2\text{O} \cdot \text{mL}^{-1} \cdot \text{s}$), 13 ($1.26 \pm 0.23 \text{ cmH}_2\text{O} \cdot \text{mL}^{-1} \cdot \text{s}$), and 15 ($1.29 \pm 0.22 \text{ cmH}_2\text{O} \cdot \text{mL}^{-1} \cdot \text{s}$), when compared with day 1 ($0.80 \pm 0.08 \text{ cmH}_2\text{O} \cdot \text{mL}^{-1} \cdot \text{s}$).

Fig. 1. – Baseline peripheral airways resistance (R_{pa}) in repeated wedge ($\square$; $n=6$) and repeated dry air challenge ($\circ$; $n=6$) airway of the dogs. Data are expressed as mean $\pm$ SEM. *: $p<0.05$, compared to day 1.

Peripheral airway reactivity to hypocapnia

Peak airway response to hypocapnia tended to increase with repeated DAC, but this increase was not significant in either repeated wedge or repeated DAC airways. However, the change in maximal R_{pa} ($\Delta R_{pa,max}$) from baseline in response to hypocapnia was significantly greater in repeated DAC airways. In repeated wedge lobes, R_{pa,max,CO_2} on day 1 was 2.85 ± 0.33 $\text{cmH}_2\text{O}\cdot\text{mL}^{-1}\cdot\text{s}$, and neither R_{pa,max,CO_2} nor $\Delta R_{pa,max,CO_2}$ changed over the duration of the experiment ($p=0.566$) (fig. 2). R_{pa,max,CO_2} in repeated DAC lobes was 3.04 ± 0.52 $\text{cmH}_2\text{O}\cdot\text{mL}^{-1}\cdot\text{s}$ ($n=6$) on day 1. Though R_{pa,max,CO_2} tended to increase with repeated challenge, this was not significant ($p=0.099$) (fig. 2a). However, R_{pa,max,CO_2} in the repeated DAC lobes was significantly higher on day 9 (3.29 ± 0.68 $\text{cmH}_2\text{O}\cdot\text{mL}^{-1}\cdot\text{s}$) than day 1 (2.18 ± 0.40 $\text{cmH}_2\text{O}\cdot\text{mL}^{-1}\cdot\text{s}$, $p=0.034$).

Reactivity to repeated DAC

Repeated DAC of peripheral airways resulted in an increase in the peak response to DAC. $R_{pa,max,DAC}$ on day 1 was 1.77 ± 0.27 $\text{cmH}_2\text{O}\cdot\text{mL}^{-1}\cdot\text{s}$ (11 airways in six dogs). $R_{pa,max,DAC}$ increased with repeated challenge ($p=0.0134$), and was significantly higher than day 1, on days 3 (2.14 ± 0.34 $\text{cmH}_2\text{O}\cdot\text{mL}^{-1}\cdot\text{s}$), 9 (2.31 ± 0.40 $\text{cmH}_2\text{O}\cdot\text{mL}^{-1}\cdot\text{s}$), 11 (2.40 ± 0.46 $\text{cmH}_2\text{O}\cdot\text{mL}^{-1}\cdot\text{s}$), 13 (2.26 ± 0.39 $\text{cmH}_2\text{O}\cdot\text{mL}^{-1}\cdot\text{s}$), and 15 (2.54 ± 0.45 $\text{cmH}_2\text{O}\cdot\text{mL}^{-1}\cdot\text{s}$) (fig. 3a). $\Delta R_{pa,max,DAC}$ did not change over the duration of the experiments (fig. 3b).

Effects of repeated bronchoscopy on BALF cell and mediator profiles

Repeated bronchoscopy had few effects on BALF cell and mediator profiles. Macrophage concentrations were higher in control (53.12 ± 12.22 $\text{cells}\cdot\text{mL}^{-1}$) than repeated wedge lobes (24.51 ± 6.53 $\text{cells}\cdot\text{mL}^{-1}$; $p=0.044$) (fig. 4a). $\text{PGF}_{2\alpha}$ concentrations were also higher in control BALF (36.00 ± 7.63 $\text{pg}\cdot\text{mL}^{-1}$) compared to repeated wedge BALF (21.10 ± 5.51 $\text{pg}\cdot\text{mL}^{-1}$; $p=0.044$) (fig. 5a). There were no other significant differences between control and repeated wedge BALF cells or mediators.

Fig. 2. – Hypocapnic reactivity in repeated wedge ($\square$; $n=6$) and repeated dry air challenge ($\circ$; $n=6$) of dogs. a) Peak peripheral airways resistance after hypocapnic challenge (R_{pa,max,CO_2}); and b) Peak change from baseline resistance after hypocapnic challenge ($\Delta R_{pa,max,CO_2}$). Data are presented as mean $\pm$ SEM. *: $p<0.05$, compared with day 1.

Effects of repeated DAC on BALF cell and mediator profiles

Repeated DAC resulted in granulocytic airway inflammation when compared with repeated bronchoscopy without DAC. Neutrophils (repeated DAC $6.79\pm2.11 \times 10^4$ $\text{cells}\cdot\text{mL}^{-1}$, repeated wedge $1.63\pm0.91 \times 10^4$ $\text{cells}\cdot\text{mL}^{-1}$; $p=0.045$), eosinophils (repeated DAC 17.69 ± 4.95 $\text{cells}\cdot\text{mL}^{-1}$, repeated wedge $2.20\pm0.77 \times 10^4$ $\text{cells}\cdot\text{mL}^{-1}$; $p=0.011$), and epithelial cells (repeated DAC 1.27 ± 0.43 $\text{cells}\cdot\text{mL}^{-1}$, repeated wedge $0.08\pm0.08 \times 10^4$ $\text{cells}\cdot\text{mL}^{-1}$; $p=0.011$) were increased in BALF recovered from repeated DAC airways when compared to the repeated wedge BALF (fig. 4b). Compared to the repeated wedge, BALF from repeated DAC lobes contained greater concentrations of leukotrienes (repeated DAC 101.13 ± 23.50 $\text{pg}\cdot\text{mL}^{-1}$, repeated wedge 7.52 ± 3.44 $\text{pg}\cdot\text{mL}^{-1}$; $p=0.001$), PGD_2 (repeated DAC 604.25 ± 312.76 $\text{ng}\cdot\text{mL}^{-1}$, repeated wedge 20.98 ± 12.59 $\text{pg}\cdot\text{mL}^{-1}$; $p=0.002$), PGE_2 (repeated DAC 50.91 ± 5.25 $\text{pg}\cdot\text{mL}^{-1}$, repeated wedge 30.01 ± 6.10 $\text{pg}\cdot\text{mL}^{-1}$; $p=0.011$), and $\text{PGF}_{2\alpha}$ (repeated DAC 184.76 ± 36.05 $\text{pg}\cdot\text{mL}^{-1}$, repeated wedge 21.10 ± 5.51 $\text{pg}\cdot\text{mL}^{-1}$; $p=0.008$) (fig. 5b). BALF from repeated DAC airways had significantly higher concentrations of neutrophils ($p<0.001$) and eosinophils ($p<0.001$) when compared with BALF from single

Fig. 3. – Reactivity to dry air (DAC) in repeated DAC airways (11 lobes in six dogs). Data are expressed as mean $\pm$ SEM. a) peak peripheral airways resistance after DAC ($R_{pa,max,DAC}$); and b) peak change from baseline resistance after DAC ($\Delta R_{pa,max,DAC}$). *: $p < 0.05$, compared with day 1.

DAC airways (fig. 4c). However, BALF from repeated DAC airways had significantly less PGE_2 ($p=0.038$) and $\text{PGF}_{2\alpha}$ ($p=0.0073$) when compared with BALF from single DAC airways (fig. 5c).

Discussion

This study demonstrates that dry air challenge repeated every 48 h increases R_{pa} . Baseline R_{pa} in repeated wedge airways remained remarkably constant. In contrast, repeated DAC resulted in marked increases in baseline R_{pa} . These increases in baseline R_{pa} developed gradually, and were not significantly different from the initial baseline until day 9 of this study (fig. 1). R_{pa} appeared to plateau at that point, suggesting that additional DAC would not result in greater obstruction. Whether the existing obstruction was caused by increased tonic activity of the smooth muscle, mucosal thickening, or intraluminal debris (e.g., mucus or exudate) or a combination of these factors could not be determined from this study, but speculation based on the results of these reactivity and BALF analysis will be made later in the discussion.

Peak response to hypocapnia did not change during the two week protocol in either repeated wedge or repeated DAC airways (fig. 2a). $\Delta R_{pa,max,CO_2}$ in repeated DAC

Fig. 4. – Bronchoalveolar lavage fluid (BALF) nucleated cell concentrations (mean $\pm$ SEM) from: a) control (□; n=6) and repeated wedge (▨; n=6); b) repeated wedge and repeated dry air challenge (DAC) (▧; n=10); and c) repeated DAC and single DAC (▩; n=10) airways. Macro: macrophages; lymph: lymphocytes; PMN: polymorphonuclear cells; Eos: eosinophils; Epith: epitheloid cells. *: $p < 0.05$, significantly different from graph pair.

airways was increased on day 9 (fig. 2b), but its transient nature calls into question the physiological relevance of this observation. Hypocapnia is believed to cause smooth muscle constriction by increasing the intracellular pH of the myocyte [15]. Additionally, thickening of the airway wall or intraluminal debris would serve to amplify normal smooth muscle constriction. The fact that minimal increases in reactivity to hypocapnia were measured, suggests that airway structural or intraluminal changes play a small role in the mechanical changes in this model.

Fig. 5. – Bronchoalveolar lavage fluid (BALF) eicosanoid concentrations (mean±SEM) from: a) control (□; n=6) and repeated wedge (▨; n=6); b) repeated wedge and repeated dry air challenge (DAC) (▩; n=10); and c) repeated DAC and single DAC (▤; n=10) airways. LTC₄: leukotriene C₄; PGF_{2α}: prostaglandin F_{2α}; PGE₂: prostaglandin E₂; TXB₂: thromboxane B₂; PGD₂: prostaglandin D₂. *:p<0.05, significantly different from graph pair.

The response to DAC, expressed as the peak R_{pa} after DAC, increased with repeated DAC (fig. 3a). The pattern of increase closely parallels that of baseline R_{pa} in the same airways (fig. 1), suggesting that the increased R_{pa} after DAC may merely reflect the increased baseline R_{pa} . Indeed, there was no significant increase in response to DAC when expressed as a change from baseline (fig. 3b).

Theoretically, a relative increase in baseline R_{pa} coupled with the same amount of smooth muscle shortening could result in an even greater response to an agonist due to the mathematical relationship between resistance and airway calibre. On the other hand, at least one study examining the relationship between baseline airway resistance and response to DAC found that two sequential challenges produced the same peak response, regardless of baseline [16]. Thus, based on the current data, it cannot be concluded that repetitive DAC enhances responsiveness to hyperpnoea with dry air.

Repeated DAC caused marked granulocytic inflammation of the airways (fig. 4). The magnitude of eosinophilia and neutrophilia in the BALF recovered from repeated DAC airways was much greater than that produced by a single DAC, both in this study (fig. 4c) and in previous studies [14]. Thus, it is unlikely that the DAC administered immediately before BAL in this study contributed to the granulocyte influx seen in figure 4. It is also unlikely that the inflammation found in the repeated DAC airways is due simply to the high airflows generated during DAC, as previous studies have demonstrated that the same flow rate with conditioned air reduced the postchallenge changes in R_{pa} [9, 17] and tissue histology [17]. The neutrophil and eosinophil influx in the repeated DAC airways appears to be an exacerbation of the late phase response that follows a single DAC in dogs [11]. Thus, it is concluded that repeated DAC causes eosinophilic airway inflammation that persists for at least 24 h after the last DAC. In particular, the exaggerated DAC-induced influx of eosinophils observed in this study is unique. Airway obstruction and eosinophilia are considered hallmarks of asthma in humans. The fact that these derangements can result from a stimulus analogous to that experienced by winter sport athletes is consistent with the hypothesis that repeated cold air hyperpnoea can produce asthma-like symptoms.

BALF obtained from repeated challenge airways contained increased concentrations of bronchoconstrictive mediators (fig. 5). Eicosanoid production plays an important role in the acute response to a single DAC in this dog model [14, 18, 19] and in humans [20–23]. However, increased mediator production alone may not be responsible for the increased obstruction after DAC, as mediator concentrations in BALF from repeated DAC airways were lower than those found in BALF obtained after a single DAC (fig. 5c). The fact that the post-DAC BALF mediator concentrations are lower after repeated DAC (compared to a single DAC) suggests either decreased capacity for mediator production in airways repeatedly challenged with dry air, decreased airway sensitivity to the dry air stimulus, or a decrease in the effectiveness of the stimulus due to the protective effects of intraluminal mucus.

Granulocytes, macrophages, mast cells, and bronchial epithelial cells are potential sources of eicosanoid mediators in BALF [24]. It seems unlikely that neutrophils and eosinophils were responsible for the eicosanoid production measured after DAC because their concentration in BALF recovered from repeated DAC airways (fig. 4) increased in the face of decreased postchallenge mediator production (fig. 5). Macrophage and mast cell numbers in BALF recovered from single DAC and repeated DAC airways were not different, suggesting that differences in

post-DAC eicosanoid concentrations were not attributable to differences in these cells. It is possible that there are fewer ciliated epithelial cells in the airways after repeated DAC, as epithelial repair after a single DAC requires >24 h to occur [25]. Thus, if the bronchial epithelium is the primary source of post-DAC eicosanoid production, then the reduced eicosanoid concentrations in a repeated DAC BALF (as compared to the BALF recovered after a single DAC) may reflect the accumulation of mucosal injury.

Post-DAC inflammation may be responsible for the increased baseline *R_{pa}* found in this study, as both neutrophils and eosinophils have been implicated in the development of airway obstruction. In a canine model of ozone-induced airway inflammation, neutrophil depletion blocked the development of airway obstruction [26]. This finding is not consistent in all studies, as others have reported dissociation between airway neutrophilia and airway obstruction [13, 27, 28]. On the other hand, eosinophils produce various cationic proteins that in other animal models produce airway obstruction through a variety of mechanisms [29]. Further study will be required to clarify the role of these cells in this model of hyperpnoea-induced airway inflammation and obstruction.

In summary, this study demonstrates that repeated dry air challenge of canine peripheral airways results in airway inflammation and progressive increases in baseline resistance. Because this model shares many similarities with cold-air hyperpnoea in humans, this study suggests that repeated episodes of cold air hyperpnoea cause airway injury and inflammation, and contributes to the development of airway obstruction. These data also support the hypothesis that repeated cold air hyperpnoea contributes to the development of asthma.

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