Effect of training and recovery on airway inflammation in an animal model of ‘ski asthma’

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Abstract
Repeated strenuous exercise while breathing cold air is believed to induce chronic airway inflammation and hyperreactivity, a condition referred to in humans as ‘ski asthma’. However, the time course of development and resolution of ski asthma is unknown. We have previously shown that multi-day aerobic exercise induces airway inflammation and hyperreactivity in racing sled dogs. In the present study, a similar group of subjects was examined at multiple times during training to test the hypothesis that ski asthma spontaneously resolves during seasonal detraining, but is re-induced during training in the cold weather. At the beginning of training, bronchoalveolar lavage fluid (BALF) from detrained elite sled dogs (n = 16) had higher concentrations of lymphocytes (median 53.63 vs. 8.30 cells µl⁻¹) and neutrophils (median 23.03 vs. 1.10 cells µl⁻¹) compared with normal laboratory dogs (n = 5). However, there was no significant effect of training on BALF nucleated cell concentrations from exercised sled dogs (n = 11) compared with sedentary sled dogs (n = 8). In contrast to our hypothesis, our data support the contention that cold weather exercise-induced airway inflammation can persist through seasonal detraining, but that routine training does not cause significant worsening of the condition.

Keywords: Exercise; bronchitis; sled dog

Introduction
‘Ski asthma’ is a syndrome of airway inflammation and hyperresponsiveness that is commonly found in human athletes who routinely perform strenuous exercise in cold weather¹⁻³. It is suspected that during cold weather strenuous exercise, the increased volume of inspired air, combined with the cold temperature and low water content of the air, exceeds the ability of the upper airways to warm and humidify the air. As a result, air that is unconditioned relative to core conditions (body temperature, 100% relative humidity) reaches the lung periphery, leading to loss of heat and water from airway surfaces that are not normally exposed to such processes⁴. If these losses are of sufficient magnitude, mucosal damage results, triggering inflammation and abnormal peripheral lung mechanics⁵.

We recently reported the characterization of a naturally occurring animal model of ski asthma in racing sled dogs⁶. In that report, we demonstrated that dogs competing in a 10-day, 1100-mile endurance race across central Alaska had airway inflammation exceeding that found in similarly trained dogs that had been rested for at least 3 weeks. Thus, we showed that it was possible (at least in racing sled dogs) to induce or exacerbate airway inflammation with cold weather exercise. Furthermore, given that the airway cytology of the sled dogs was quite similar to that reported in human ski asthma⁵, we provided partial validation of racing sled dogs as a suitable model of this syndrome.

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These findings in turn raised a logical question: for how long does cold weather exercise-induced airway inflammation persist in the absence of repeated injury? In the present study, we used a group of racing sled dogs to test the hypothesis that cold weather exercise-induced airway inflammation resolves during the detraining period between competitive seasons, and is re-induced with the renewed onset of cold weather exercise.

### Experimental design

All procedures were approved by the Oklahoma State University Institutional Animal Care and Use Committee according to the principles outlined in the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. Two groups of dogs were studied: 19 racing Alaskan sled dogs (11 males and eight females, between 4 and 8 years old, ranging in body weight from 22 to 33 kg) that had been rested from training for approximately 4 months throughout the Alaskan summer, and five sedentary control dogs (mixed-breed hounds, male, 2 years old, body weight range 19–22 kg) that had never been exposed to sustained exercise or frigid conditions. In the first experiment, all dogs were anaesthetized with propofol (7 mg kg\(^{-1}\) intravenously) and placed on positive-pressure ventilation using a piston-type ventilator. Tidal volume was set at 17 ml kg\(^{-1}\) body weight, and ventilator rate was adjusted to maintain end-tidal CO\(_2\) between 3.5 and 4.5%. A 5 mm OD fibre-optic bronchoscope was advanced into a lower airway. Bronchoalveolar lavage was performed by infusing and aspirating three sequential, 20 ml boluses of phosphate-buffered saline. The recovered fluid was pooled, and an aliquot was placed in a tube containing NaEDTA for cytological analysis. Determination of the total nucleated cell concentration in bronchoalveolar lavage fluid (BALF) samples was made within 24 h by use of a haemocytometer. Differential cell counts were determined using cytocentrifuged slide preparations stained with a modified Wright–Giemsa stain.

In the second experiment, the 19 sled dogs were randomly assigned to exercise or non-exercise groups. The exercise group commenced training for competitive endurance racing in a fashion typical of the sport, whereas the non-exercise dogs remained housed together with the exercise group, but did not participate in any training. BALF was obtained and analysed as in the first experiment from both groups, 2 months and 4 months into the training season. The final examination occurred during January when the dogs were considered fit for endurance competition. In the month immediately preceding the last examination, typical ambient conditions were \(-29^\circ\text{C}\), and the exercise group was completing 25–40-mile training runs four or five times weekly.

As the data from the first experiment were not normally distributed, total and differential cell concentrations were compared using the Mann–Whitney rank sum test. Analyses of variance were performed with PC SAS Version 8.2 (SAS Institute, Cary, NC). Each dog was assessed over time, so intra-dog covariance structures were adjusted using repeated-measures techniques in a REPEATED statement in PROC MIXED. Three covariance structures were examined for each response variable: autoregressive period 1, unstructured and variance components. The structure that yielded the lowest Akaike information criterion (AIC) was adopted for the analysis. For BALF macrophage concentration, the autoregressive covariance structure gave the lowest AIC. The unstructured covariance was utilized for both BALF lymphocyte and eosinophil concentrations. For BALF neutrophil concentrations, the variance component covariance structure was used. The simple effects of treatment given time point and time point given treatment were assessed with a SLICE option in an LSMEANS statement. All responses in time points 2 and 3 were divided by the initial value (time point 1) to give percentage of baseline. Any comparison with a \(P\)-value less than 0.05 was considered significant.

### Results

All dogs tolerated anaesthesia and bronchoalveolar lavage well, with no subsequent evidence of pulmonary compromise and only minimal (< 48 h) interruption in training. Subjective impressions of bronchoscopic appearance were unremarkable in all dogs at all times. We were unable to obtain sufficient BALF from three sled dogs during the first examination due to equipment malfunction. The BALF volume recovered from sled dogs was significantly less than that recovered from laboratory dogs \(31.4 \pm 14.0 \text{ vs. } 45.0 \pm 3.1 \text{ ml, } P = 0.001\).

Trained sled dogs had significantly greater concentrations of inflammatory cells in their airways than untrained control dogs, even after 4 months of rest (Fig. 1). Sled dogs had approximately six times more lymphocytes (median 53.63 \text{ vs. } 8.30 \text{ cells } \mu\text{l}^{-1}, \text{ } P = 0.001) and 20 times more neutrophils (median 23.03 \text{ vs. } 1.10 \text{ cells } \mu\text{l}^{-1}, \text{ } P = 0.0074). There was a strong trend towards increased BALF concentration of eosinophils in the sled dogs (median 15.95 \text{ vs. } 3.5 \text{ cells } \mu\text{l}^{-1}, \text{ } P = 0.0683). There were no significant differences in the concentration of BALF macrophages.

Subsequent training of a randomly selected subset of these sled dogs throughout the winter had minimal impact on BALF cytology, compared with their non-exercised kennel mates (Figs 2–4). There was a
significant decrease over time of BALF macrophage concentrations from the exercised dogs ($P = 0.0092$), and of BALF lymphocyte concentrations from both exercise ($P = 0.0044$) and non-exercise ($P = 0.0200$) dogs throughout the winter. There was no effect of treatment (exercise vs. non-exercise) at any time point.

**Discussion**

In our previous study comparing sled dogs immediately after sustained exercise with rested, trained sled dogs, we demonstrated that sustained cold weather exercise could cause the influx of leucocytes into the peripheral airways\(^6\). In doing so, we confirmed the findings of a canine laboratory model of exercise-induced asthma\(^7\)–\(^9\), as well as the suspicions raised in human studies\(^1\)–\(^3\), that the combination of hyperventilation and extremely cold inspired air can cause peripheral airway inflammation. In our current study, we failed to support the first part of our hypothesis (that cold weather exercise-induced airway inflammation resolves during seasonal detraining) in that, even after 4 months of detraining, residual airway inflammation remained. These data complement recently reported measurements of airway mechanical properties that showed airway obstruction and hyperresponsiveness to aerosol histamine in rested sled dogs\(^10\). The character of the inflammation after 4 months of rest (increased BALF lymphocytes and neutrophils) was slightly different from that previously reported in racing sled dogs immediately after sustained multi-day strenuous exercise (increased BALF macrophages, lymphocytes and eosinophils\(^6\)). Part of the discrepancy can probably be explained by the statistical power of the respective studies. In the current study, the difference in BALF eosinophil concentrations displayed a strong trend towards significance ($P = 0.0683$) and in the earlier report, the exercise-induced increase in BALF neutrophils displayed a strong trend towards significance ($P = 0.055$). Thus, the only qualitative difference between the airway inflammation immediately after sustained exercise and after sustained rest is the apparent normalization of BALF macrophage concentrations.

The persistence of the airway inflammation (as well as the mechanical abnormalities) is somewhat surprising, considering the hypothesized aetiology behind

![Fig. 1](image1.png)  
Leucocyte concentrations in bronchoalveolar lavage fluid (BALF) from laboratory dogs ($n = 5$) and rested sled dogs ($n = 19$). Macro – macrophages; Lymph – lymphocytes; PMN – neutrophils; Eos – eosinophils. *$P < 0.05$.

![Fig. 2](image2.png)  
The effect of endurance training on macrophage concentrations in bronchoalveolar lavage fluid (BALF) in (a) exercising sled dogs ($n = 11$) and (b) non-exercising sled dogs ($n = 8$). Combined datasets were evaluated using two-way repeated-measures analysis of variance. There was an effect of time in exercise ($P = 0.0092$) but not in non-exercise ($P = 0.5311$) dogs. There was no effect of treatment at any time point.

![Fig. 3](image3.png)  
The effect of endurance training on lymphocyte concentrations in bronchoalveolar lavage fluid (BALF) in (a) exercising sled dogs ($n = 11$) and (b) non-exercising sled dogs ($n = 8$). Combined datasets were evaluated using two-way repeated-measures analysis of variance. There was an effect of time in exercise ($P = 0.0044$) and in non-exercise ($P = 0.0200$) dogs. There was no effect of treatment at any time point.
the inflammation of ski asthma. During strenuous cold weather exercise, it is believed that loss of water from the peripheral airways results in transient hyperosmolality\textsuperscript{4,11}, which in turn activates airway epithelial cells to release pro-inflammatory cytokines (interleukin-8 and RANTES (regulated upon activation, normal T-cell expressed and secreted))\textsuperscript{12,13}. Mast cell-derived products may also be involved, because mast cells have been shown to degranulate in response to hyperosmolar stimuli\textsuperscript{14} as well as in the canine laboratory model of ski asthma\textsuperscript{15}. However, the cellular influx has been shown to be at least partially with 3 weeks of rest in trained sled dogs\textsuperscript{16} and to resolve completely within 1 week of rest in the canine laboratory model of ski asthma\textsuperscript{16}. The relevant difference between the inflammation produced by the canine laboratory model and the racing sled dog model may lie in the longer duration of challenge and maintenance of airway inflammation in the latter. However, a specific biochemical explanation for greater persistence of the airway inflammation in the sled dogs is unknown.

Although our data suggest that recovery from the airway inflammation of ski asthma requires more than 4 months, we cannot say based on our data what the minimum recovery time should be. Unfortunately, our study was not designed to extend this rest period, since after our examination in July ambient conditions at the kennel gradually became colder and the dogs resumed athletic training. Although an absolute determination of the amount of rest needed to completely resolve the airway inflammation would be of scientific value, such information would be of less practical value since typically competitive kennels can afford to rest the dogs for only 4 months before resuming training. Nevertheless, examination of the BALF data from the sled dogs suggests that recovery continued into the winter (Figs 2–4), hinting that initial training at least does not interfere with recovery. It is important to note that although the non-exercise sled dogs were not participating in formal exercise training, their exuberant nature combined with outdoor housing may have resulted in occasional modest cold air hyperventilation. Thus, we cannot state categorically that the period from July until the conclusion of the study was completely free of airway challenge in the non-exercised dogs, nor can we be certain that recovery would not have proceeded faster in the complete absence of vigorous activity.

An additional weakness of our current experimental design is related to possible breed differences between our control laboratory hounds and our sled dogs. Alaskan sled dogs are not a specific, American Kennel Club-recognized breed. Rather, they are typically a mix of northern breeds characterized by having thick, double-layered coats. Although the majority of sled dog kennels do not attempt to preserve or include specific breeds, there is clearly a strong contribution of Siberian Husky to the Alaskan sled dog gene pool, and we cannot exclude the possibility of this influence contributing to the development of exercise-induced airway inflammation. In fact, Clercx et al.\textsuperscript{16} have reported Husky-type dogs to be disproportionately represented in their initial report of eosinophilic airway disease in dogs. However, the breeding programmes of racing kennels are rigorous processes of imposed selection, with the overriding criterion being athletic performance. If conditions that impaired cardiopulmonary performance had a genetic component, they would be systematically eliminated from the gene pool of racing Alaskan Huskies in just a few generations. Thus, we believe it is unlikely that the differences described in this report are due to genetic factors.

It is interesting that, despite the finding of increased leucocyte concentrations in sled dog BALF, we did not detect any visual abnormalities during bronchoscopy. We believe the discrepancy stems from two factors. First, bronchoscopic evaluation is confined to the larger central airways, whereas BALF is obtained from the lung periphery, and thus the explanation may simply be that the abnormalities are localized to the lung periphery. In this regard, it is important to note that the airway mechanical abnormalities documented in these dogs are also principally located in the distal airways\textsuperscript{10}. Second, bronchoscopy provides only indirect and insensitive evidence of inflammation (increased mucus secretion and/or decreased
clearance), and thus is unlikely to be as sensitive as direct measurement of inflammatory cells. Such findings have also been reported in horses with mild, non-infectious lower airway inflammation. Thus, while bronchoscopy is certainly a good screening tool for detecting lower airway inflammation, we believe that actual demonstration of increased inflammatory cells through examination of airway secretions should be considered the gold standard for confirming peripheral lung airway inflammation such as occurs in cold weather exercise-induced airway injury.

Our data also failed to support the second part of our hypothesis, as there was no apparent de novo induction of airway inflammation with the onset of training. We find two possible explanations for these findings. The first is that exercise while breathing cold air does not induce airway inflammation. We believe that the scientific literature, including our own previous studies, suggests this explanation is unlikely. The alternative explanation, and one that we feel is supported by the scientific literature, is that the magnitude of airway injury that occurs during training is insufficient to induce substantial airway inflammation, particularly when cast against a background of pre-existing airway inflammation. A clue as to the magnitude of challenge required to induce the full spectrum of airway inflammation can be found by comparing the exercise challenges of training versus competition. In the previous study of sled dogs immediately after competition, the exercise challenge was the Iditarod sled dog race. In this race, teams of dogs average 110 miles per day for 10 consecutive days, in conditions ranging from relatively warm (−5 to 5°C) in the first few days to as cold as −30°C for the majority of the race. A typical strategy is to provide the dogs with equal run/rest periods during the race. There are no published estimates of the relative minute ventilation during this exercise, but because a dog relies heavily on the respiratory tract to dissipate excess heat and there is considerable metabolic heat production in racing dogs (~12 000 kcal day⁻¹), it is likely that minute ventilation is substantially increased for 12–14 h daily for the duration of the race. In contrast, during early training conditions are comparably mild (~5 to 5°C) and distances are considerably shorter (40–50 miles per day, 4 days per week), even at the peak of training. We have previously shown that the induction of airway inflammation is linearly related to the magnitude of the challenge. Our current results suggest that the magnitude of challenge resulting from routine training is insufficient to overcome the inherent variability of our measurements (although in the case of neutrophils, a slight increase in subject number may prove statistically useful). Future studies will involve challenges of greater magnitude and larger subject numbers to help confirm what appears to be an interesting trend.

While the causes of the airway inflammation in ski asthma appear to be coming into focus, the consequences are far less certain. The dogs in this study and in the previous study of dogs after Iditarod competition did not have any clinical signs of airway disease or pulmonary compromise. In fact, the subjects of the Iditarod sled dog study finished in the top ten places of arguably the most elite competitive event in the sport, and therefore had passed numerous veterinary examinations to be permitted to continue racing. These observations follow the general pattern of the reports of ski asthma in humans, in which the most profoundly affected subjects are often Olympic-calibre athletes, frequently from some of the most dominant teams in their sports. Thus, there is little current evidence that the airway inflammation leads directly to reduced pulmonary function. However, it is possible that the negative effects of cold air-induced airway inflammation are more subtle. In particular, the apparent activation and degranulation of mast cells secondary to airway hyperosmolarity may preferentially release pro-inflammatory mediators specific for the TH2 phenotype. As a result, subsequent response of the airways to antigen may be shifted towards humoral immunity, in particular the production of immunoglobulin E. The effects of prolonged cold air-induced airway inflammation on pulmonary immunity warrant further investigation.

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References


