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Evidence supporting exercise-induced pulmonary haemorrhage in racing greyhounds

TS Epp[1,*], B Szladovits[1], A Buchannan[1], L Gates[1], P McDonough[2], DJ Padilla[1], J Smart[3], HH Erickson[1] and DC Poole[1]

[1]Department of Anatomy and Physiology, Kansas State University, Manhattan, Kansas 66506, USA
[2]Department of Kinesiology, University of Texas at Arlington, Arlington, Texas 76019, USA
[3]Veterinary Consultant for the National Greyhound Racing Association, 619 Woodland Drive, Washington, Kansas 66968, USA
* Corresponding author: tepp@vet.k-state.edu

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Abstract

Exercise-induced pulmonary haemorrhage (EIPH) is a major health concern in performance horses, but the presence and severity of this condition in racing greyhounds has received little attention. While equids and greyhounds share many physiological attributes, there are important structural and functional differences that may help protect greyhounds from EIPH. We tested the hypothesis that greyhounds performing a simulated 503 m race would experience EIPH and that the time course of recovery would be similar to the horse, even though the severity or relative extent as indexed by the concentration of red blood cells [RBCs] in bronchoalveolar lavage (BAL) fluid would be lower in comparison with that demonstrated previously in horses. Greyhound dogs ($n = 6$) raced on two occasions (separated by 7 weeks) and BAL was performed 1 week before, 2 h after and each week for 4 weeks following each race to examine the [RBC], concentration of white blood cells [WBCs], WBC differentials and haemosiderophages in the lungs. Racing increased 10 min post-exercise venous blood [lactate] to $18.6 \pm 0.4\,\text{mmol}\,\text{l}^{-1}$. No epistaxis or pink froth was observed at the nose or mouth of any of the dogs. The [RBC] in the BAL fluid was increased significantly 2 h post-race (baseline $= 109.6 \pm 11.7 \times 10^3$; post-race $= 292.3 \pm 69.9 \times 10^3$ RBC ml^{-1} BAL fluid, $P < 0.05$) and returned to baseline 1 week post-race ($149.2 \pm 46.2 \times 10^3$ RBC ml^{-1} BAL fluid, $P > 0.05$ *versus* baseline). The number of haemosiderophages was not different for any of the measurement periods. The [WBC] in the BAL fluid decreased from baseline and race values at 2, 3 and 4 weeks post-exercise (all $P < 0.05$). Alveolar neutrophil concentrations were also decreased from baseline and immediate post-race values for 4 weeks post-race. The increased [RBC] in the BAL fluid post-exercise is consistent with the presence of EIPH in these greyhounds. However, the relative extent of EIPH in greyhounds (as indexed by [RBC] in the BAL fluid), as compared with that in the horse, was mild, and the lack of elevation of WBC suggests that, unlike their equine counterparts, inflammatory airway disease was absent.

Keywords: EIPH; racing greyhounds; bronchoalveolar lavage; inflammatory airway disease

Introduction

Exercise-induced pulmonary haemorrhage (EIPH) has been reported to occur in nearly all horses[1–4], as well as camels[5], humans[6,7] and dogs[8,9] following strenuous exercise. Although this condition has been well documented in the Thoroughbred horse since the 17th century[10] and researched extensively, only two preliminary reports provide evidence that greyhounds experience EIPH[8,9].

In horses, the aetiology of EIPH is multifactorial, with high pulmonary artery pressures[11,12], large airway pressure swings[3,13–22], inflammatory airway disease (IAD)[1,23–29] and ground impact forces[30–32] hypothesized to be the major contributing factors. The exercise response of greyhounds is physiologically similar to that of the horse in many ways. The Thoroughbred horse and racing greyhound are both exceptional sprinters[33–35] (*c.* $18\,\text{m}\,\text{s}^{-1}$) with massive cardiac outputs[33–36] (0.6 and $1.01\,\text{kg}^{-1}\,\text{min}^{-1}$ in horse and greyhound, respectively)

and exceptional elevations in packed cell volume (PCV; 65 and 66% in horse and greyhound, respectively)[34,35,37,38] during maximal exercise. The pulmonary capillary transmural pressure required for rupture in the canine pulmonary capillary (66–70 mmHg)[39,40] measured *ex vivo* is similar to the mean pulmonary artery pressure (Ppa) reported for the greyhound during sub-maximal exercise at 11 m s^{-1} (*c.* 55 mmHg)[41]. Thus, it is likely that the greyhound approaches or exceeds this 'threshold for capillary rupture' during maximal exertion. One important difference between canine and equine athletes is that horses are obligate nasal breathers during exercise. This, in combination with extraordinarily high airflow rates and a long trachea (where length of airway is proportional to resistance of airway according to Poiseuille's law), causes the development of exceptionally sub-atmospheric extravascular pulmonary pressures, which summate with the intravascular pressures to create the very high transmural pressures measured in the horse. As the greyhound is not an obligate nasal breather and during exercise achieves far lower peak airflows than the horse through a much shorter, though narrower, trachea, it is possible that the fall in alveolar pressure[42–44] is less extreme and this therefore reduces the potential for EIPH in this species.

While pulmonary capillary transmural pressures may be lower in greyhounds than in horses, the pulmonary capillary blood–gas barrier is weaker in the dog[40]. Specifically, when subtracting oesophageal pressure (Pes) from mean Ppa to estimate pulmonary artery transmural pressure, a value of greater than 65 mmHg (close to canine stress failure threshold) is estimated during sub-maximal exercise. Thus, it should not be surprising that preliminary data suggest that greyhounds do experience EIPH during maximal sprint exercise[8,9].

This investigation was designed to determine whether greyhounds run over the standard 503 m course demonstrate a significant increase in [RBC] in the BAL fluid, and, if present, outline the timeline for resolution. Specifically, we tested the hypothesis that greyhounds would exhibit a significant increase in [RBC] in the BAL fluid, though to a lesser relative extent in comparison with the horse, but following a similar pattern and time course of recovery (i.e. disappearance of [RBC], elevation of white blood cells (WBC) and haemosiderophage emergence, peak and decline) to that documented for the horse.

Methods

Animals

Six healthy greyhound dogs that had been raced on the track were acquired for this study. The group consisted of four intact females and two intact males ranging in age from 2 to 4 years and weighing 29.0 ± 1.2 kg. They were housed in a temperature-controlled building (70–74°F with 30–70% humidity). They had a 0.3 m^2 area inside their pen with free access to a 74 m^2 outdoor sand run. Indoor conditions were on a 12-h light:12-h dark cycle. The dogs were fed Iams (Iams Mini Chunks®, Iams Company, Dayton, OH, USA) adult dog food (minimum of 26% crude protein, minimum of 15% crude fat, maximum of 5% crude fibre and maximum of 10% moisture) once daily in the morning and had free access to water. The greyhounds were current on vaccinations including distemper virus, adenovirus, parainfluenza virus, parvovirus and *Bordetella bronchiseptica*, as well as being on monthly Heartgard Plus. All procedures used were approved by the Kansas State University Animal Care and Use Committee.

Animal preparation

The greyhounds were fed 3 h before estimated race time. The dogs were transported in a climate-controlled dog trailer *c.* 81 km to a local training track.

Exercise protocol

Greyhounds were divided into two heats with three dogs in each heat and run on the track in a race-simulated timed race (503 m distance) around a track, chasing a lure. After 9–10 min of completing the race, a venous blood sample was obtained from the jugular vein with a 22 gauge 1 in. needle attached to a 3 cc heparinized syringe with a stopcock for post-exercise plasma lactate analysis. Greyhounds were not formally exercised post-race, but did have access to their runs. A second identical race (race 2) was performed 2–3 weeks after RBC and WBC counts had stabilized near baseline levels (*c.* 7 weeks post-race 1).

Bronchoalveolar lavage

The initial baseline BAL was performed and cultures obtained after the dogs had not been formally exercised or raced for 1–2 weeks after their acquisition by Kansas State University. One week after the baseline lavage, the initial post-race lavage was performed *c.* 2 h post-exercise under general anaesthesia, this sampling procedure examined the number of RBC per millilitre of BAL fluid (which is considered to be a reflection of the amount of blood in the airways post-exercise), which we took as an indirect measure of the relative extent of EIPH[2,3,8,9,17–19,22,23,45–49]. Briefly, after intramuscular pre-medication with atropine (0.025 mg kg^{-1}), morphine (0.25 mg kg^{-1}) and diazepam (0.2 mg kg^{-1}) had taken effect, the dogs were induced with intravenous propofol (6 mg kg^{-1}; PropoFlo™, Abbott Laboratories, North Chicago, IL, USA), intubated and placed on supplemental oxygen. Dogs remained in sternal recumbency for the

procedure. They were maintained with an intravenous infusion of propofol ($0.4\,mg\,kg^{-1}\,min^{-1}$) in order to allow the collection of the BAL fluid samples. For health and safety reasons, heart rate, systemic arterial pressure, oxygen saturation, respiratory rate/character, mucous membrane colour and depth of anaesthesia were monitored by a technician. After the animals were stabilized under anaesthesia, a flexible modified foal stomach tube[50] with a diameter of 16-F (Argyle stomach tube, Sherwood Medical, Company, St Louis, MO, USA) was wedged in a sub-segmental bronchus of the caudal dorsal lung lobe after insertion through the endotracheal tube. The location of the distal end of the BAL tube was assumed to be the dorsocaudal lung region from the work of Hawkins[50], but it was verified radiographically on all six greyhounds before and after one of the lavage trials. Before insertion, the distal end was transected with a sterile blade above the most proximal fenestration (*c.* 6 cm from the distal end). A sterile hand-held metal pencil sharpener was used to round the distal end of the tube and create a slight taper to enhance formation of a tight seal when the tube was wedged in a bronchus of the caudal lung lobe. During insertion once resistance was felt, an attempt was made to gently withdraw and reposition the catheter, ensuring placement in the most distal airway possible. A Christmas tree adapter was placed on the proximal end of the BAL catheter. Following placement of the catheter, a 50 ml aliquot of sterile isotonic saline (0.9%), warmed to body temperature, was slowly infused. After a short delay (*c.* 2 breaths), the fluid was aspirated with gentle suction while chest copage was performed bilaterally. This procedure was repeated thrice, equivalent to a dose of $5\,ml\,kg^{-1}$ of instilled fluid per dog (*c.* 125–150 ml of total per dog). Supplemental oxygen was administered after the procedure until extubation and thereafter provided by mask until the dog recovered. The dogs were monitored every 4–6 h after the procedure for signs of respiratory distress, pale mucous membranes or prolonged capillary refill time, and twice daily for the next 48 h for signs of respiratory distress, cough, lethargy or loss of appetite. The BAL samples were placed on ice immediately after collection and remained chilled throughout the analysis. The BAL fluid was centrifuged (10 min at $600 \times g$; TJ-6 Table Top Centrifuge, Beckman Instruments, Inc., Palo Alto, CA, USA), after which the supernatant was removed via gentle suction and the pellet resuspended in sterile 0.9% saline. RBC and WBC were then quantified manually with standard haemocytometer counts (Fisher Scientific, Pittsburgh, PA, USA; Nikon, Inc., Instrument Group, Garden City, NY, USA), expressed as cells ml^{-1} BAL fluid, and differential slides were made (Cytospin 2, Shandon, Pittsburgh, PA, USA) and evaluated. These slides were stained with a diff-quick stain (Hema-Stain, Fisher Scientific) that stains cells similar to the Wright–Giemsa stain. Cytospin slides were also stained with Perl's Prussian Blue Stain as previously described by Meyer *et al.*[2] and the number of haemosiderophages was determined from the entire slide that had uniform number of cells (1×10^6) per slide.

After the initial post-race lavage, the lavage procedure was then performed at 1-week intervals to determine the time course of recovery from EIPH. There was a 2-week interval between the fourth-week post-race lavage and baseline 2. The post-race 2 lavage was done 1 week after the second baseline in a similar manner to race 1.

Blood analysis

Following venous blood withdrawal (jugular vein *c.* 10 min post-race) into plastic heparinized syringes, blood samples were placed immediately on ice. Within 2 h of the experiment, plasma lactate was quantified using a Nova Stat M blood–gas analyzer (Nova Biomedical, Waltham, MA, USA). This machine was calibrated according to the manufacturer's standards immediately prior to running the samples.

Statistics

Differences in measured variables were analysed using a mixed effects model where time is a fixed effect, and dog and dog × time are random effects. When significant differences were found, a least-square means post-hoc test was used to determine where the differences existed. The Statistical Analysis System Program 9.1.2 statistical package (SAS Institute, Inc., Cary, NC, USA) was used to analyse the data. Significance was accepted at the $P \leq 0.05$ level.

Results

The percentage of BAL fluid recovery was similar for all lavages (race and resting lavages) and ranged from 59 to 68% with an average of 63 ± 1% (Table 1). Cultures obtained from the first lavage were negative for any pathogens.

Red blood cell concentrations in the lavage fluid

The greyhounds exhibited a significant increase in the [RBC] in the BAL fluid ($P = 0.025$) when determined after both race 1 ($P = 0.036$) and race 2 ($P = 0.016$). Baseline and post-race 1 values (mean ± SE) were $144.5 \pm 21.9 \times 10^3$ and $313.8 \pm 70.5 \times 10^3$ RBC ml^{-1} BAL fluid, respectively. Baseline and post-race 2 values (mean ± SE) were $74.7 \pm 13.1 \times 10^3$ and $270.7 \pm 120.1 \times 10^3$ RBC ml^{-1} BAL fluid, respectively. The mean levels of RBC ml^{-1} in the BAL fluid for the baselines and races were consistent (no significant differences existed between baseline 1 *versus* baseline

Table 1 Bronchoalveolar lavage (BAL) data

Variable	Baseline	Race	1 WPR	2 WPR	3 WPR	4 WPR
Total RBC ml^{-1} BALF	109 600 ± 11 700	292 300 ± 69 900*	149 200 ± 46 200†	79 000 ± 15 500†	94 900 ± 31 100†	109 900 ± 57 700†
Total WBC ml^{-1} BALF	134 000 ± 14 900	141 000 ± 19 900	122 000 ± 32 100	84 000 ± 10 900†*	89 700 ± 14 500†*	75 200 ± 3900†*
% Return BALF	66.3 ± 3.5	60.7 ± 3.1	63.6 ± 4.4	59.5 ± 3.4	60.3 ± 6.0	66.7 ± 4.3
Macrophages (cells ml^{-1} BALF)	93 100 ± 9330 (70.7 ± 3.3)	95 600 ± 14 200 (68.6 ± 2.9)	95 500 ± 4960 (78.3 ± 4.1)*†	64 600 ± 2090 (76.5 ± 2.5)†	66 900 ± 3100 (74.5 ± 3.5)	50 000 ± 3960 (66.5 ± 5.3)
Neutrophils (cells ml^{-1} BALF)	17 000 ± 3250 (12.6 ± 2.5)	16 400 ± 4020 (11.3 ± 2.1)	7310 ± 2670*† (6.0 ± 2.2)	7740 ± 1470*† (9.2 ± 1.7)	7920 ± 1260*†(8.8 ± 1.4)	9770 ± 2990*†(13.0 ± 4.0)
Lymphocytes (cells ml^{-1} BALF)	19 900 ± 3840 (14.3 ± 1.7)	26 600 ± 5080 (18.7 ± 2.4)	18 700 ± 3600 (15.3 ± 3.0)	11 800 ± 2040 (14.0 ± 2.4)	14 810 ± 2240 (16.5 ± 2.5)	13 800 ± 2220 (18.3 ± 3.0)
Eosinophils (cells ml^{-1} BALF)	4410 ± 2920 (2.4 ± 1.7)	2250 ± 1660 (1.6 ± 1.0)	610 ± 610 (0.5 ± 0. 5)	279 ± 279 (0.3 ± 0.3)	153 ± 153 (0.2 ± 0.2)	1630 ± 1630 (2.2 ± 2.2)
Mast Cells (cells ml^{-1} BALF)	214 ± 163 (0.2 ± 0.1)	0 ± 0 (0.0 ± 0.0)	0 ± 0 (0 ± 0)	144 ± 144 (0.2 ± 0.2)	0 ± 0 (0 ± 0)	0 ± 0 (0 ± 0)
Haemosiderophages	41 ± 10	64 ± 38	108 ± 40	80 ± 36	21 ± 4	77 ± 38

Data represent averaged bronchoalveolar lavage (BAL) variables for six greyhounds that were lavaged 2 h, and weekly, after two separate 5/16 mile races. The differential counts are presented with the actual number of white blood cells (WBCs) by type (i.e. macrophage, neutrophils, lymphocytes, eosinophils and mast cells) on the top and the differential percentage directly below it. Haemosiderophage numbers represent the number on a slide with 1×10^6 WBCs per slide. Time periods across the top of the table are abbreviated as follows: baseline, resting BAL; race, 2 h post-race BAL; 1 WPR, 1 week post-race BAL (resting); 2 WPR, 2 weeks post-race BAL (resting); 3 WPR, 3 weeks post-race BAL (resting); 4 WPR, 4 weeks post-race BAL (resting). *Significant difference ($P < 0.05$) from race. †significant difference ($P < 0.05$) from baseline;

2 or race 1 *versus* race 2), so these two data points were averaged (baseline = $109.6 \pm 11.7 \times 10^3$ RBC ml^{-1} BAL fluid and race = $292.2 \pm 69.9 \times 10^3$ RBC ml^{-1} BAL fluid) for ease of presentation and interpretation (Fig. 1). The number of RBC ml^{-1} BAL fluid had returned to baseline by 1 week post-race (149.2 ± 46.2 RBC ml^{-1} BAL fluid; Fig. 1).

Additional bronchoalveolar lavage fluid analysis

As there was no significant difference between the two races, the baseline and race lavages were averaged as for the RBC data. There was no change in the [WBC] 1 week post-race, but there was a significant decrease from baseline 2–4 weeks post-race (Fig. 2; Table 1). Haemosiderophage counts for these periods were quite variable but did not change significantly from baseline (Table 1). Despite the absence of an inflammatory response, there were significant differences in the differential counts. Specifically, the concentration of neutrophils in the BAL fluid was decreased from baseline during the entire 4 weeks post-race ($P < 0.05$, Table 1). As a result of these decreased neutrophils, the relative percentage of alveolar macrophages (occasionally containing vacuolation) increased at 1 week post-race and remained significantly elevated for 2 weeks, returning to baseline levels at 4 weeks post-race (Table 1).

Blood lactate, rectal temperature and performance times

Venous blood [lactates] were 19.2 ± 0.4 and 18.0 ± 0.5 mmol l^{-1} ($P > 0.05$) for 10 min post-race samples for races 1 and 2, respectively, with the average being 18.6 ± 0.4 mmol l^{-1}. Ten minutes post-race, rectal temperatures were 106.0 ± 0.3 and 104.5 ± 0.4°C for races 1 and 2, respectively ($P > 0.05$), with the average being 105.2 ± 0.3°C. Race times were 32.1 ± 0.3 and 32.6 ± 0.4 s for races 1 and 2, respectively ($P > 0.05$, average 32.2 ± 0.1 s; a competitive time for the lower-end greyhound would be 31.0–31.5 s).

Discussion

The principal original findings of this investigation are that: (1) These greyhound dogs run under simulated race conditions at 503 m experienced an increase in [RBC] in post-race BAL, and that increase was of a lower magnitude compared with that described previously in horses[2,17–20]. (2) Recovery from elevated [RBC] in the BAL fluid post-race in greyhounds followed a similar pattern to horses when looking at [RBC], in that the numbers of RBC ml^{-1} BAL fluid returned to baseline levels within 1 week post-race. In contrast to the horse, this did not result

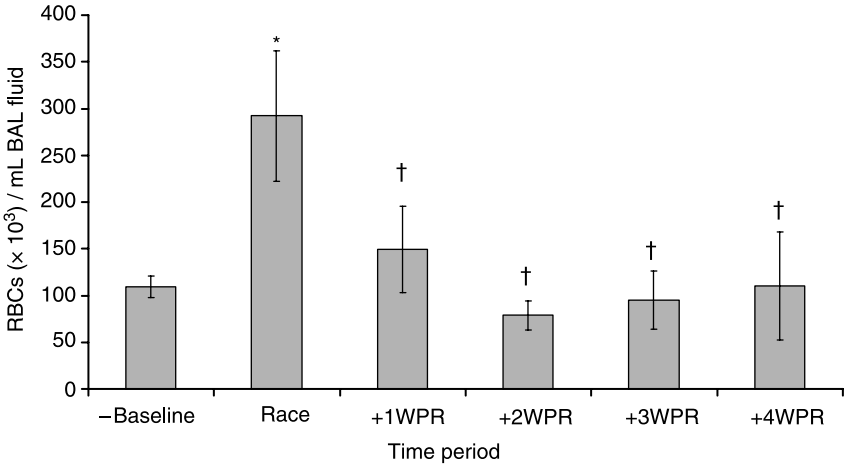

FIG. 1 Exercise-induced pulmonary haemorrhage (EIPH) in greyhounds as assessed by bronchoalveolar lavage (BAL; $n = 6$) performed before and after a 5/16 mile race. EIPH is expressed as the number of red blood cells (RBCs) per millilitre of BAL fluid. Data are presented as mean ± SE of the average of the baselines (1 and 2) and the races (1 and 2). Time periods on the abscissa are abbreviated as follows: baseline, resting BAL; race, 2 h post-race BAL; 1 WPR, 1 week post-race BAL (resting); 2 WPR, 2 weeks post-race BAL (resting); 3 WPR, 3 weeks post-race BAL (resting); 4 WPR, 4 weeks post-race BAL (resting). Lavages were performed 1 week apart. *Significant increase in EIPH (increased number of RBC) from baseline; †significant decrease in the number of RBC per millilitre of BAL fluid compared with immediately post-race values

in prolonged elevation of haemosiderophages or an extended inflammatory response in the lung.

Comparison with the current literature

A very limited amount of information exists concerning EIPH in racing greyhounds (see preliminary reports)[8,9]. King[8,9] reported significant EIPH from the dorsocaudal lung region in greyhounds racing 503 m, ranging from 1 to 71 million RBC ml^{-1} BALF with an occasional dog displaying endoscopic evidence of EIPH. The data from the current study are in agreement with the preliminary studies by King *et al.*[8,9], suggesting that greyhounds may demonstrate pulmonary haemorrhage during exercise. However, the [RBC] detected in the BAL fluid in the current study was much lower than that found by King[8,9]. Between-study differences in the [RBC] of the BAL fluid post-race may be due to methodological factors including time from run to lavage (20 min in King *et al.*[8,9] studies *versus* 2 h in present study), induction of iatrogenic haemorrhage, lung differences (right *versus* left lung predominance in greyhounds) and pre-selection of dogs for the study (certain populations of animals may potentially be more prone to severe EIPH).

The greyhounds in the current study demonstrated an increase in [RBC] in the BAL fluid detected 2 h post-race, which returned to baseline levels within 1 week post-exercise as reported for the horse[2].

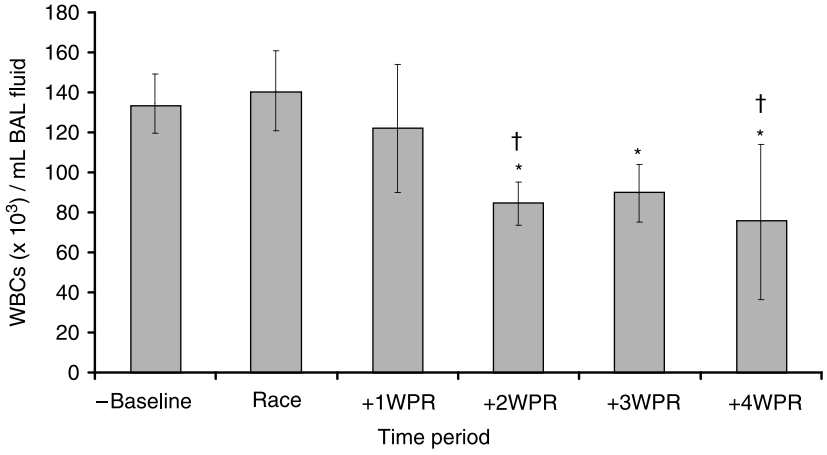

FIG. 2 White blood cells (WBCs) per millilitre of BAL fluid measured in the bronchoalveolar lavage (BAL) fluid 2 h after greyhounds ran a 5/16 mile race and at weekly intervals thereafter (abscissa labelled as for Fig. 1). Data are presented as mean ± SE of the average of the baselines (1 and 2) and the races (1 and 2). Lavages were performed 1 week apart. No significant differences occurred from baseline to the race. *Significant decrease in WBCs per millilitre of BAL fluid post-race; †significant decrease in the number of WBCs per millilitre of BAL fluid from baseline ($P < 0.05$)

However, the amount detected in the airways is far lower than values measured typically in the horse (EIPH = 4–64 × 10^6 RBC ml^{-1} BAL fluid)[19,22,23,45–47,50]. Haemosiderophages were also examined in the study of Meyer *et al.*[2] as an indicator of past pulmonary haemorrhage[1,2,51,52]. In horses, haemosiderophages are commonly around 7% at baseline and increase significantly (10–20%) 1 week post-exercise, remaining elevated for 3–4 weeks post-exercise. This equine profile is in contrast to the extremely low, almost undetectable levels of haemosiderophages that we found in greyhound dogs. Due to extremely sparse numbers of haemosiderin-laden macrophages, it was impossible to obtain a percentage of haemosiderophage count for the dog slides. Therefore, the total number of haemosiderophages per million cells (entire slide) was counted to increase the accuracy of comparison within dogs and among BAL periods. The small amount of blood detected in our study did not induce an increase in haemosiderophages 1–3 weeks post-exercise as observed previously in the horse[2]. Moreover, alterations in the haemosiderophage counts for the greyhounds were erratic with respect to pre- and post-race evaluations, and this profile may potentially be explained by mild iatrogenic bleeding initiated during the lavage procedure. These data are in agreement with King *et al.*'s[9] observation that 7 out of 10 dogs showed evidence of haemosiderin deposits within alveolar macrophages indicating past haemorrhage.

IAD in the horse is hypothesized to contribute to the initiation and severity of EIPH as well as being a response to the condition[1,24–28]. A long-standing inflammatory reaction to blood within the alveoli results in a self-perpetuating phenomenon of gradually worsening haemorrhage as horses continue to train and race[24,53]. However, in the racing greyhound, a much lower [RBC] in BAL was detected after a race, which may not be sufficient to overwhelm pulmonary defences and these RBCs may be cleared too rapidly to invoke any prolonged inflammatory response. It has been shown in the horse that alveolar macrophage function (phagocytosis and oxidative burst) becomes suppressed for 2–3 days when challenged by alveolar haemorrhage[24,54–60] as a consequence of the limited ability of alveolar macrophages to effectively metabolize/detoxify the iron in RBC[61,62]. It is also possible that IAD itself may not induce the long-term pulmonary damage that exacerbates EIPH in the horse[27,29]. The present investigation did not find an elevated WBC concentration in the lavage fluid at baseline (in comparison with the literature)[63–72] nor following racing. However, an unexpected decrease occurred in the WBC concentration at weeks 2, 3 and 4 after racing. A decrease in the concentration of neutrophils was detected for 4 weeks post-race, which follows the WBC concentration and corroborates the absence of an inflammatory response to the mild haemorrhage. It is possible that weekly use of propofol (an anaesthetic drug with anti-inflammatory properties) could aid in masking a mild inflammatory response[73,74]. We did note a significant rise in the percentage of macrophages (not actual numbers) from 1 to 3 weeks post-race that resulted from a significant decrease in neutrophils, since the actual concentration of macrophages remained unchanged. These results are also in agreement with EIPH studies of King *et al.*[8,9] but at variance with the equine literature where the WBC concentration typically becomes elevated after an episode of EIPH and either remains elevated or decreases slowly, depending on the independent contribution from IAD. Again, the greyhound dogs in the present investigation did not appear to have true IAD at any time (i.e. elevation in WBC concentration with concurrent increase in macrophages, neutrophils and/or lymphocytes). Other conditions cause inflammation in the lower airways of dogs, but are totally unrelated to the current study and include 'ski asthma' in Alaskan sled dogs, allergic bronchitis and eosinophilic airway disease[75]. When looking at WBC concentration and differentials from BAL fluid, much variation exists in the canine literature. In support of our conclusion that a post-race inflammatory response was not evident, the WBC concentration and differentials obtained herein fall within the published ranges[63–72].

Mechanisms of exercise-induced pulmonary haemorrhage

EIPH has been studied most extensively in the Thoroughbred horse and several mechanisms have been proposed to contribute to the pathophysiology of EIPH in that species. In order of potential importance as considered in the literature, these include elevated pulmonary arterial pressures[11,12,76], airway contributions[3,13–22], IAD[1,23–29], blood viscosity[77] and locomotory impact forces[30–32].

Greyhounds may be expected to demonstrate EIPH as they have a number of physiologic attributes in common with the horse. Both species are exceptional sprinters[33,34], have large heart-to-body weight ratios[78–81], large contractile spleens (high PCV)[34,37,38,77], high pulmonary vascular pressures during exercise[12,41] and very high maximal oxygen consumptions[33,35,36,82,83] for their body size. In addition, greyhounds have a thinner blood–gas barrier than the horse that requires a smaller transmural pressure gradient to cause capillary rupture[3,39,40,84], as reported by Birks *et al.*[84] (dog, 0.795 ± 0.084 μm; 66 mmHg and horse, 0.930 ± 0.044 μm; 70–100 mmHg) or potentially similar when reported as harmonic mean thickness (dog 0.509 ± − 0.002 × 10^{-4} cm and pony 0.511 ± 0.031 × 10^{-4} cm) by Weibel *et al.*[85]. Greyhounds have high cardiac outputs[33,34,36] that play a large role

(in combination with their highly elevated PCV) in generating both exceptional levels of oxygen uptake[33,36] and potentially high pulmonary arterial pressures during maximal exercise. Based on pulmonary artery pressures of greyhounds measured during sub-maximal exercise (55 mmHg at 11 m s^{-1}), peak exercising pressures at speeds approaching 18 m s^{-1} might well be much higher.

Notwithstanding the above, these are features specific to the greyhound that may protect this animal from the extensive rupture of the blood–gas barrier observed in the horse. Specifically, the dog is not an obligate nasal breather and has a much shorter trachea than the horse (airway resistance is proportional to the length of the airway and inversely proportional to the radius to the fourth power), thus, depending on the airflows achieved and airway(s) resistance, the work of breathing and pleural pressure swings generated[42] may not be as high as those seen in the horse. Saibene *et al.*[43] found that dogs during exercise experienced a pronounced dilation of the respiratory tract, which reduces airflow resistance. The horse also has a very stiff chest wall that limits the thoracic displacement to ventilation during exercise[86] and mandates a proportionally greater diaphragmatic displacement. Consequently, horses may be predisposed to more sub-atmospheric intrapulmonary pressures and also shear forces that damage the dorsocaudal lung lobes. The more compliant chest wall of the dog may well limit these effects if it permits lateral chest wall expansion during exercise. Findings support the hypothesis that impact forces are a significant risk factor for EIPH in horses[30–32]. However, these forces will be reduced in the dog in proportion to their body frames and weights, along with the consideration that they are not carrying a rider and saddle. Lastly, the severity of EIPH may depend on the duration over which the threshold capillary transmural pressure is exceeded[87]. Thus, the shorter distance (greyhounds, 503 m *versus* Thoroughbred horses, 1609–2416 m) and reduced time (25–35 s for greyhounds *versus* 1–2 min for horses) at which maximal effort is sustained by the greyhound *versus* the Thoroughbred horse may help constrain the magnitude of EIPH. However, racing Quarter Horses can demonstrate extreme EIPH in races of similar distance to greyhounds[88], and Thoroughbreds themselves can evidence epistaxis breaking from the starting gate.

Methodological considerations

Bronchoalveolar lavage (BAL) has been utilized for a little over a decade in horses to evaluate the concentration of RBC present in the BAL fluid post-exercise[51], and is frequently referred to in the equine[2,3,17–19,22,45–49] and human literature[7,89] to quantify the degree or severity of EIPH. An advantage of BAL is that there is not a precise window of time within which BAL has to be performed, as is the case with endoscopy[2,90]. In addition, there is less chance of missing blood due to the timing of post-exercise endoscopy or failure of blood movement into the larger airways where it can be visualized and graded[2,48,91]. Even though King *et al.*[8,9] did visualize small amounts of bleeding in two dogs with endoscopy, the relatively low [RBC] in BAL observed post-race in the current study would not likely have been observed with endoscopy[1,2,51,92]. It is possible that these low [RBC] found in these greyhounds may be partially explained by the 75- to 90-min delay in performing the lavage procedure. The 2 h post-race lavage was chosen *versus* 30 min post-race as is conventional in the equine literature[2,3,17–19,22,45–48] and similar to the King studies[8,9] due to the increased safety of transporting the dogs back to the veterinary teaching hospital for the procedure instead of anaesthetizing the dogs at the track. It has been demonstrated that smaller RBC counts may be observed with large time lapses post-race, as in the study of Valdez *et al.*[49] where BAL was performed 12–18 h post-race. On the other hand, the level of bleeding could truly be less in maximally exercised dogs and may be further lowered by the extended time between race and lavage.

BAL has been considered to be the most accurate[2,51] and sensitive[91] technique available to detect and quantify the level of pulmonary haemorrhage. However, an obvious limitation of equating [RBC] with severity is its reliability, because this assumes that RBC counts in the lavage fluid are proportional to the severity of haemorrhage, which has not been definitively shown[91]. Despite this inability to absolutely quantify the amount of blood in the lung, utilization of the BAL technique and enumeration of [RBC] offers the best estimate of the extent of EIPH aside from removing the entire lung for whole lung lavage[19], which is impossible for repeatedly quantifying the severity of EIPH in living horses[91]. Furthermore, techniques such as scintigraphy and endoscopy have greater limitations to accuracy and sensitivity and limit quantitative and objective assessment[19]. What has been shown, at least in horses where EIPH has been studied extensively, is that a good correlation exists between BAL cytology and histopathology in horses with EIPH[49,92,93].

It also has been shown in the horse that blind placement of the BAL tube typically results in wedging it in the dorsocaudal region[94] of the lung where the majority of EIPH occurs[25,26,51,94]. Due to physiological similarities between the dog and the horse, as well as the preliminary data gathered with the bronchoscope in the greyhound[8,9], the dorsocaudal region was the site the authors chose to examine, realizing that the

differences in canine physiology and anatomy could potentiate bleeding in other locations of the canine lung. Unpublished radiographic data (TS Epp, AM Buchannan, L Gates, DC Poole and HH Erickson) from our laboratory suggest a similar caudal lung lobe placement (seven out of seven dogs) when a BAL tube is passed blindly (five right caudal lung lobes *versus* two left caudal lung lobes) in the greyhound. Even though side predominance is not statistically significant, this is in agreement with Hawkins *et al.*[50], who radiographically demonstrated right caudal lung lobe predominance when a BAL tube was blindly placed (seven dogs right *versus* two dogs left). Therefore, it may be of importance that King *et al.*[8,9] showed that the left lung may haemorrhage to a greater degree than the right one, which is in contrast to what has been observed in the horse[2,95]. It is also important to keep in mind that the greyhounds utilized in this study were not selected based upon a prior history of severe EIPH, as is often the case in horse studies[3,19,22,23,45–47]. In addition, though these greyhounds raced giving maximal effort, their racing times were 0.7–1.0 s slower than many competitive greyhounds, which may have impacted the relative extent of EIPH.

BAL is becoming more popular as a diagnostic tool for pulmonary disorders in dogs. However, BAL results can be difficult to interpret. Disadvantages or problems potentially encountered in dogs (but not in horses) include the possibility that alveoli are non-uniform and intermittent collapse occurs, not allowing lavage fluid into the distal airways as well as an increased rate of mucociliary clearance in the dog *versus* the horse (3.3 *versus* 2 cm min^{-1})[96–98]. In addition, there may be mild trauma to the airways obtained while trying to ensure wedging of the tube[50,67,68] and there is the potential for airway collapse[68] distal to the catheter if too much suction is inadvertently applied, resulting in possible trauma and a smaller recovery volume. In the current study, precautions were taken to minimize this trauma (i.e. use of sufficiently rigid tubing so that resistance could be felt and to prevent bending upon itself, but flexible enough to pass through the airways and minimize trauma with careful passage; continuous gentle suction applied with cessation upon first indication of negative pressure during aspiration of sample). It is not possible to differentiate whether RBC detected in the lung lavages originated from EIPH or from iatrogenic bleeding, but [RBC] are consistent across samplings from horses at rest[2]. In the current study, baseline 1, weeks 1, 2, 3 and 4 post-race, as well as baseline 2 were performed 1 week apart with consistent [RBC], which would be unexpected if the techniques were inducing iatrogenic haemorrhage. There is no qualitative colour evidence of haemorrhage, but baseline [RBCs] are present in horses not exercised (at least 10 days to 2 weeks)[2,48,99], so it is not surprising that baseline levels are also evident in the canine. The [RBC] in the BAL fluid has been used in horses as an index to indicate the relative extent of EIPH with various therapeutic interventions and alterations in exercise intensity and workload. The results and conclusions have been consistent, predictable and reproducible in multiple investigations from multiple laboratories. Lower [RBCs] are observed during submaximal (trotting) exercise *versus* maximal effort[48], at different exercise intensities[3], and during inclined *versus* flat running[46]. To date, BAL has demonstrated a reduction in EIPH with nasal strips[17–19,22,49], furosemide[18,19,22], and concentrated equine serum[23]. An increased [RBC] in the BAL fluid has been demonstrated with nitric oxide inhibition using L-Name[100] and inhaled nitric oxide[45]. Therefore, it follows from the equine data that the significant increase above baseline, which was observed in the [RBC] 2 h post-exercise in the dog, would be consistent with the occurrence of EIPH.

Considerable variation and lack of standardization exist in the precise volume of lavage fluid infused, the technique employed (i.e. the number of aliquots, interval between instillation and aspiration, lobes lavaged)[71,101] and the processing of fluid (i.e. centrifugation, washing and counting)[63,65,67,71]. The techniques utilized in this study were chosen after careful perusal of the available literature as well as some trial and error, and were found to combine the most consistent results with the least amount of iatrogenic trauma possible. Finally, depending specifically on volume and the number of aliquots, normal numbers and differential percentage of WBC in the lavage fluid are less defined than those available for equids[102]. Variations in the WBC counts as well as the differential cell counts[65,71] may possibly be due to lavaging of different levels (i.e. bronchial *versus* alveolar)[72], different concentrations obtained[50,63] and the lung lavaged (i.e. left or right)[8].

Implications of increased [RBC] in the BAL fluid of greyhounds post-exercise

The current investigation provides evidence that the greyhound exhibits a significant increase in [RBC] in the BAL fluid following maximal running, which is consistent with EIPH, albeit at very low levels compared with equids. However, one must use caution when directly comparing the [RBC] in the BAL fluid from equids to canids because any differences in pulmonary anatomy/physiology and the relative size of the scope or BAL tube to the airway could alter the level of haemorrhage detected in response to exercise. On the other hand, if these greyhounds are typical of

the entire racing population, it does not appear that the consequences of that bleeding are as pernicious and clinically worrisome as is the case for the horse. This conclusion is based on the fact that the levels of WBC, haemosiderophages and differential counts did not indicate a prolonged response to the blood (i.e. large inflammatory response and elevations in haemosiderophages over time) as is typically seen with the horse. Therefore, these dogs may not suffer the long-term lung damage and performance deficits created by inflammation and fibrosis. However, validation of this hypothesis will necessitate post-mortem pulmonary anatomical studies similar to those done in horses. Such studies are necessary to confirm or refute the notion that EIPH is not of major clinical significance in the greyhound as it is in the horse.

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Evidence supporting exercise-induced pulmonary haemorrhage in racing greyhounds. T S Epp, B Szladovits, A Buchannan, L Gates, P McDonough, D J Padilla, J Smart, H H Erickson, D C Poole. Published: 1 February 2008. by Cambridge University Press (CUP). in Comparative Exercise Physiology. Comparative Exercise Physiology , Volume 5; doi:10.1017/s147806150891906x. Publisher Website. Google Scholar. Keywords: Induced Pulmonary / pulmonary haemorrhage / exercise induced / Racing Greyhounds / Supporting Exercise. Scifeed alert for new publications. Never miss any articles matching your research from a Exercise-induced pulmonary hemorrhage: where are we now?Â Investigations using BAL indicate that hemorrhage occurs in almost all horses in racing or training,2,60,61 and BAL cytological findings cohere closely with clinical disease, pulmonary histopathology and endoscopic evidence of EIPH.57â€“60 Concerns regarding BAL most often include the need for rapid access to the horse post-race, the need for sedation (eg, 10â€“20 Î¼g/kg intravenous detomidine.