Effects of training and strenuous exercise on hematologic values and peripheral blood leukocyte subsets in racing sled dogs

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Exercise training and exercise itself are known to cause predictable physiologic changes with corresponding alterations in peripheral blood components in many species. In some instances, these changes reflect appropriate physiologic adaptations to the demands of exercise, in others, they reflect pathophysiologic abnormalities arising from the stress of exercise. Regardless, changes may be sufficiently large to necessitate the use of unique reference ranges when evaluating results of hematologic analyses in athletic animals.

Some of the changes in hematologic values associated with exercise and exercise training, such as a decrease in RBC count secondary to plasma volume expansion, are advantageous, in that they facilitate the specific physiologic requirements of exercise. Others, such as neutrophilia, are not necessarily advantageous but are consistently expected and, thus, may represent the norm for an athletic population. Finally, other changes may represent the presence of disease processes common to athletes and, although expected, should nevertheless be considered abnormal.

The present study was designed to determine expected ranges for hematologic values in racing sled dogs under conditions specifically associated with exercise training and extended-duration exercise. We wanted to determine not only the effects of exercise itself, but also the effects of the nutritional and environmental conditions that make such exercise possible. Furthermore, we wanted to identify possible explanations for deviations from reference values for the general population of dogs. Specifically, the purpose of the study reported here was to determine the effects of training and sustained submaximal exercise on hematologic values in racing sled dogs.

Materials and Methods
The experimental protocol was reviewed and approved by the Oklahoma State University Institutional Animal Care and Use Committee. Thirty-nine mixed-breed racing sled dogs from a single large racing kennel in Alaska were included in the study. There were 16 females and 23 males; mean ± SD age was 3.9 ± 1.8 years. All dogs were fed a diet consisting of variable combinations of commercial kibble and fish and meat scraps. The diet was individually adjusted to maintain body weight during training and exercise. All dogs received routine vaccinations and internal parasite control medications. None of the dogs had any clinically apparent disease at the time blood samples were collected.
Blood samples were collected from all dogs prior to the beginning of training for competition. All dogs had undergone a 4-month period of rest prior to collection of blood samples.

Additional blood samples were collected from 19 of the 39 dogs (6 females and 13 males; mean ± SD age at the time of blood sample collection, 4.9 ± 1.8 years) after a full season of exercise training consisting of approximately 7 months of progressive increases in speed and distance designed to allow dogs to compete in races of up to 1,100 miles. Blood samples were collected 1 week prior to a multiday endurance race; dogs had not been exercised for at least 48 hours prior to sample collection. Samples were collected only from dogs that had achieved a level of training such that they were considered capable of successfully completing an 1,100-mile race.

A final set of blood samples was collected from 9 of the 19 dogs from which a second sample had been obtained (3 females and 6 males; mean ± SD age at the time of blood sample collection, 4.7 ± 1.9 years). These blood samples were collected within 2 hours after dogs completed a 10-day, 1,100-mile race. Samples were not collected from dogs that started the race but failed to complete it for any reason.

In all instances, blood samples were collected by means of jugular venipuncture into 3-mL glass tubes containing 7.5% EDTA. Samples were chilled and shipped overnight on the day of collection to the Washington State University College of Veterinary Medicine, where they were immediately processed. A CBC, differential cell count, and flow cytometry for leukocyte surface antigens were performed. Complete blood counts were performed with automated equipment.²

Flow cytometry—Leukocytes were separated from RBCs by means of density-gradient separation, as described.³ In brief, samples were centrifuged at 400 × g for 30 minutes in a high-density separation medium (density, 1.119) to retain granulocytes and mononuclear cells at the interface. Cells were collected and subjected to several cycles of low-speed centrifugation (200 × g for 8 minutes) to reduce platelet contamination. Cells were then suspended in phosphate-buffered saline solution containing 20% acid citrate dextrose at a concentration of 2 × 10⁸ cells/µL.

Fifty microliters of cells (approx 10⁸) was added to each well of 96-well, conical-bottom microtitration plates to which mouse anti-canine CD4 and anti-canine CD8 antibodies had been added. Specificity of the antibodies against canine CD4 and CD8 has been reported previously.⁴ Cells were incubated on ice for 15 minutes, then subjected to 3 cycles of washing by means of centrifugation (700 × g for 3 minutes) and were then resuspended in fresh phosphate-buffered saline solution containing acid citrate dextrose. Cells were incubated an additional 15 minutes with Cy-5 conjugated goat anti-mouse IgG₁ and fluorescein-conjugated goat anti-mouse IgM.⁵ Cells were then washed twice in phosphate-buffered saline solution containing acid citrate dextrose and fixed in 2% buffered formaldehyde and stored in the dark in a refrigerator until examined.

Flow cytometry was performed with a flow cytometer⁶ equipped with argon and red lasers and standard software.⁷ Electronic gates were placed on lymphocytes, monocytes, and granulocytes to exclude noise at the time of data acquisition. Because CD4 is commonly expressed on neutrophils from dogs, cells in the granulocyte gate were excluded when determining percentages of CD4⁺ and CD8⁺ lymphocytes in cell preparations. Data were analyzed with standard software. Negative controls included samples processed with only the anti-mouse antibodies and were prepared and analyzed concurrently to confirm the absence of nonspecific binding. Previous work by one of the authors (WCD) has shown stable expression of canine leukocyte surface antigens for up to 3 days with refrigeration.

Statistical analysis—Because blood samples were not collected from all dogs at all time points, a randomized, incomplete-block model was used for analysis of hematologic data. Least squares mean values were calculated with adjustment of raw mean values for imbalance and missing data. Values obtained prior to and after training were compared by means of ANOVA followed by pairwise t tests to determine the effects of training on hematologic values. Values obtained after training and after completion of the 1,100-mile race were compared to determine the effects of sustained strenuous exercise on hematologic values. All analyses were performed with standard software.⁸ Values of P < 0.05 were considered significant.

Results

Training caused a modest increase in total WBC count, primarily as a result of a significant increase in neutrophil count that was partially offset by a significant decrease in lymphocyte count (Figure 1). Exercise caused a further substantial increase in total WBC count, again largely because of a further significant increase in neutrophil count. No significant differences in band neutrophil count were observed between the 39 dogs from which a second sample had been obtained (3 females and 6 males; mean ± SD age at the time of blood sample collection, 1.9 ± 1.8 years) after a 7-month training regimen (untrained; n = 39), after completion of the training regimen (trained; 19), and after completion of an 1,100-mile race (exercised; 9). PMN = Neutrophil count. Lymph = Lymphocyte count. Mono = Monocyte count. Eos = Eosinophil count. For each cell type, columns with different letters were significantly (P < 0.05) different from each other. Neutrophil count is plotted against the left y-axis; counts for all other cell types are plotted against the right y-axis. Error bars represent SD.

![Figure 1](image-url)
identified among values obtained prior to training, after training, and after exercise. Eosinophil count after exercise was significantly lower and monocyte count after exercise were significantly higher than values obtained after training, but values obtained after training were not significantly different from values obtained after exercise identified among values obtained prior to training, after training, and after exercise. Eosinophil count after exercise was significantly lower and monocyte count after exercise were significantly higher than values obtained after training, but values obtained after training were not significantly different from values obtained after exercise.

**Discussion**

Results of the present study suggested that training and exercise induced changes in several hematologic values in racing sled dogs. In particular, both training and exercise caused significant decreases in PCV and hemoglobin concentration and significant increases in total WBC count. On the other hand, training and exercise were not found to have significant effects on absolute numbers or fractions of CD4+ or CD8+ lymphocytes, other than a significant increase in the fraction of CD8+ lymphocytes associated with training.

Racing sled dogs are not a specific breed but necessarily possess certain phenotypic traits, such as athletic capacity and a dense, protective hair coat, that are maintained through selective breeding. Thus, our sample population was not representative of the entire population of domestic dogs, and this potential source of bias must be considered when evaluating the results of the present study. However, hematologic values obtained prior to training were consistent with values for dogs in general that have been published previously, suggesting that the selection process used to produce racing sled dogs has not resulted in a shift in basic cellular hematologic.

Exercise in other species produces predictable changes in circulating leukocytes. For the most part, similar changes were observed in the present study. However, the mechanisms postulated for the changes described in other species did not appear to apply to the changes obtained in the present study. For example, in people, exercise-induced neutrophilia is believed to be caused by an increase in circulating concentrations of catecholamines, and the neutrophilia is rapidly reversed as the catecholamine concentration again decreases. In contrast, it is unlikely that this mechanism affects circulating neutrophils in racing sled dogs. However, this does not exclude the possibility that catecholamines play a role in the exercise-induced neutrophilia observed in these dogs.

**Table 1—Effect of training and exercise on lymphocyte subsets in racing sled dogs.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Untrained</th>
<th>Trained</th>
<th>Exercised</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+ (%)</td>
<td>28.90 ± 10.53</td>
<td>32.53 ± 7.17</td>
<td>33.67 ± 6.33</td>
</tr>
<tr>
<td>Absolute count (cells/µL)</td>
<td>651.0 ± 372.8</td>
<td>501.5 ± 276.1</td>
<td>485.2 ± 224.4</td>
</tr>
<tr>
<td>CD8+ (%)</td>
<td>22.41 ± 7.13</td>
<td>27.05 ± 7.74</td>
<td>29.56 ± 7.52</td>
</tr>
<tr>
<td>Absolute count (cells/µL)</td>
<td>490.4 ± 285.4</td>
<td>416.5 ± 230.4</td>
<td>433.0 ± 182.4</td>
</tr>
<tr>
<td>CD4+CD8+ ratio</td>
<td>1.51 ± 1.16</td>
<td>1.30 ± 0.39</td>
<td>1.24 ± 0.51</td>
</tr>
</tbody>
</table>

**Table 2—Effect of training and exercise on RBC and platelet variables in racing sled dogs.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Untrained</th>
<th>Trained</th>
<th>Exercised</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (cells x 10^6/µL)</td>
<td>7.17 ± 0.60</td>
<td>6.53 ± 0.80</td>
<td>5.10 ± 0.64</td>
</tr>
<tr>
<td>Hgb (g/dL)</td>
<td>17 ± 1</td>
<td>18 ± 1</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>49 ± 4</td>
<td>44 ± 4</td>
<td>30 ± 4</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>35 ± 1</td>
<td>35 ± 1</td>
<td>37 ± 1</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>24 ± 1</td>
<td>24 ± 1</td>
<td>24 ± 1</td>
</tr>
<tr>
<td>Platelets (platelets/µL)</td>
<td>252,100 ± 101,701</td>
<td>334,684 ± 73,456</td>
<td>335,889 ± 67,933</td>
</tr>
<tr>
<td>MPV (fl)</td>
<td>16.11 ± 1.36</td>
<td>9.37 ± 2.35</td>
<td>9.41 ± 0.71</td>
</tr>
</tbody>
</table>

**MCH = Mean corpuscular volume, MCHC = Mean corpuscular hemoglobin concentration, MCH = Mean corpuscular hemoglobin, MPV = Mean platelet volume.** See Table 1 for remainder of key.
can explain the training-induced neutrophilia seen in the present study, in that dogs had not been exercised for at least 48 hours prior to collection of blood samples after the training period. Furthermore, in a previous study, we were not able to document increased concentrations of catecholamine metabolites in urine samples collected from racing sled dogs after exercise. Similarly, exercise-induced lymphopenia in people is widely believed to be a result of exercise-induced release of cortisol. Although we have previously found that serum cortisol concentration was increased in sled dogs after an 1,100-mile race, a significant decrease in lymphocyte count was seen after training in the present study, and serum cortisol concentrations in trained sled dogs prior to exercise are within reference limits. It is possible that the observed decrease in lymphocyte count in the present study was a result of transient episodes of hypercortisolemia resulting from training, with the resulting lymphopenia requiring greater time to resolve than the clearance of the excess cortisol. This possibility requires further study.

Values for leukocyte subsets in the present study, including values obtained prior to training, were strikingly different from those reported previously for healthy dogs. In 1 study, for instance, mean CD4+ fraction for adult (mean age, 3.7 years) mixed-breed dogs was 42%, whereas mean fraction prior to training for dogs in the present study was 28.90%. Mean CD8+ fraction for mixed-breed dogs in the previous study was similar to mean fraction prior to training for dogs in the present study (22.41%), with the result that the CD4+ : CD8+ ratio for sled dogs in the present study was lower than that reported for healthy mixed-breed dogs (1.74). Definitive assessment of immune function in sled dogs will require additional studies of, in particular, vaccine responses and the quantity and functional quality of induced antibodies.

There are conflicting data in the literature on the effects of training on RBC indices in sled dogs. Kronfeld et al reported an increase in PCV in response to aerobic training in sled dogs, whereas Reynolds et al and Querengaesser et al did not identify significant changes in PCV in response to training. Differences in training regimen among these studies do not seem to account for the disparate results, as dogs in the studies by Kronfeld et al and Querengaesser et al were trained for 20 to 24 weeks and accumulated similar total training distances (400 to 600 miles). These previous studies stand in stark contrast to the more recent training strategies reflected in the present study. Current competitors in endurance sled dog racing typically train their dogs for as long as 26 weeks, and dogs may have accumulated as many as 2,000 training miles by the end of the racing season. Although precise training data were not available for dogs in the present study, it was likely that their training was substantially more intense than that in previous studies, and this may account for the differences between results of the present study and results of these previous studies.

Conflicting information also appears in the literature regarding the effect of sustained exercise on RBC indices. Hinchcliff et al, for instance, did not identify any effect of exercise on PCV of dogs entered in the Yukon Quest sled dog race, whereas Burr et al reported a 10% decrease in hemoglobin concentration during the Iditarod sled dog race and a 14% decrease in PCV during a 170-mile exercise. Differences between results of the present study and results of these previous studies may stem from methodologic differences. In the study by Hinchcliff et al, samples were not collected from dogs before or during the first half of the race. Thus, the apparent lack of an effect of exercise may have been due to different rates of decrease during extended exercise, with more substantial losses occurring during the early part of the exercise period and losses tapering off with continued exercise. Such a pattern is supported by the results of Burr et al, in that greater losses were found after a shorter exercise challenge. However, the overall intensity of the exercise challenge in the previous studies was considerably less than that in the present study. Dogs in the study by Burr et al finished the Iditarod in 12 days 16 hours, whereas dogs in the present study finished the same course in just over 9 days. Thus, the greater decreases in various RBC indices reported in the present study may be related to the more intense exercise challenge. It is also important to note that our method of subject selection may have introduced a bias that underestimated the overall effect of exercise on PCV and hemoglobin concentration because individual dogs with larger decreases might have been expected to be dropped from competition because of poor performance secondary to anemia.

There are 2 likely causes for the decreases in RBC count associated with training and exercise in the present study: plasma volume expansion and gastrointestinal tract losses. Endurance exercise training of dogs has been shown to cause a 13% to 27% expansion of plasma volume with a concurrent reduction in PCV. Although we did not measure plasma volume in the present study, such a change in plasma volume would explain the small change in RBC count seen during training, suggesting that the training-induced decrease in PCV and related variables may have simply been a result of water retention.

Plasma volume expansion may also explain the observed effects of exercise on PCV, but this was difficult to determine with the current study design. An exercise-induced reduction in PCV of approximately 1 percentage point per day of racing has been reported previously, and we are confident that this is a reliable population benchmark reflecting current competitive exercise intensities. Studies of trained human endurance athletes have reported plasma volume expansion as a result of prolonged marathon-type exercise in addition to the plasma volume expansion resulting from training, but this effect is not realized until sufficient time has elapsed to recover from the dehydration resulting from exercise itself. However, these studies have used the Dill and Costill formulas for calculating plasma volume. The validity of this technique is dependent on the total RBC mass being unchanged, and studies of exercising humans and racing sled dogs have demonstrated exercise-induced gastrointestinal tract bleeding. Thus, it cannot be assumed that the total RBC mass remains constant during strenuous exercise, and it is possible that reported exercise-associated changes

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in plasma volume in human endurance athletes may be due, in part, to exercise-induced gastrointestinal tract bleeding. Reliable determination of changes in plasma volume following multiday endurance exercise and, by extension, the contribution of such changes to decreases in PCV will likely require measurement of plasma volume by means of dye dilution methods. Reduced erythropoiesis is unlikely to account for the decrease in RBC count, in that given an average RBC lifespan of 110 days, even sudden complete cessation of erythropoiesis will result in a decrease of only approximately 1% of the total RBCs per day, or reduction in PCV of approximately 0.4% per day. Accelerated intravascular or extravascular RBC destruction is a possible cause of reduced RBC count. Although the former is supported by the increase in mean corpuscular hemoglobin concentration found in the present study, a high mean corpuscular hemoglobin concentration is most commonly a laboratory artifact resulting from free hemoglobin in the plasma secondary to ex vivo hemolysis. High mean corpuscular hemoglobin concentration secondary to intravascular hemolysis is normally accompanied by alterations in plasma color and hemogloburia, neither of which was observed in these dogs. A previous study reported that dogs completing an endurance race had serum bilirubin concentrations higher than the upper reference limit for dogs (3.56 mg/dL), suggesting that these dogs had a high rate of hemoglobin turnover. However, another study found low PCV with normal serum bilirubin concentrations, and we know of no likely cause or mechanism for exercise-induced accelerated RBC destruction. Thus, we believe the exercise-induced decrease in RBC count was related to blood loss. Exercising sled dogs are known to have a predisposition to gastrointestinal tract ulceration, making gastrointestinal tract bleeding a likely cause of the observed RBC loss. Methods of assessing gastrointestinal tract bleeding in sled dogs are relatively insensitive because only a small fraction of the gastrointestinal tract can be examined by means of endoscopy and detection of blood in gastrointestinal tract contents is limited to direct observation of fresh or digested blood in vomitus or feces. Although these techniques have excellent positive predictive values, their negative predictive values are likely quite low, with the results that these techniques likely underestimate the prevalence of gastrointestinal tract bleeding in sled dogs. It is noteworthy that serum protein, albumin, and globulin concentrations follow a similar pattern during exercise, providing further support for blood loss as the cause of exercise-induced RBC loss.

In summary, training- and exercise-induced changes in hematologic values for racing sled dogs in the present study were both typical and atypical of those reported for other species. Modest dilution of the blood secondary to extracellular fluid volume expansion was the likely explanation for the training-induced decrease in PCV, and acute blood loss secondary to gastrointestinal tract bleeding was likely responsible for the more dramatic decrease in PCV associated with acute exercise. In contrast, the neutrophilia and lymphopenia in these dogs, although consistent with changes reported for human athletes, occurred during training, rather than exercise, suggesting that mechanisms other than those postulated to explain findings in human athletes played a role.

References


