Muscle injury and antioxidant status in sled dogs competing in a long-distance sled dog race

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Abstract

Exercise is associated with an increase in the production of oxidants that may be instrumental in the development of exertional rhabdomyolysis. We speculated that participation in a long-distance sled race would alter antioxidant capacity of dogs, in conjunction with increases in indices of rhabdomyolysis. The objective was to determine the effect of participation in a long-distance sled dog race on antioxidant capacity and plasma creatine kinase (CK) activity in sled dogs. This was a prospective, longitudinal study on a convenience sample of 57 Alaskan sled dogs participating in a 1600 km sled dog race. Blood samples were collected before racing (31 dogs) and after racing (39 dogs) for measurement of plasma vitamin E concentration; CK, aspartate aminotransferase (AST) and caeruloplasmin (CER) activity; and red-blood-cell (RBC) glutathione peroxidase (GPX) and superoxide dismutase (SOD) activity. CER and GPX activities after the race were 26% and 14% lower, respectively, than before racing whereas CK and AST were 300% and 170% greater. There was no change in plasma vitamin E concentration or RBC SOD activity. We conclude that completion of a long-distance sled dog race involving prolonged and repeated submaximal exercise results in a reduction in enzymatic antioxidant activity in the blood of sled dogs.

Keywords: creatinine kinase; caeruloplasmin; glutathione peroxidase; superoxide dismutase; vitamin E

Introduction

Participation by Alaskan sled dogs in long-distance sled races results in significant increases in the serum activities of creatine kinase (CK) and aspartate aminotransferase (AST), as a result of leakage of the enzymes from damaged skeletal muscle cells1–4. Increases in CK correlate with increases in serum isoprostane concentration, a marker of lipid peroxidation, suggesting that free radicals produced during exercise contribute to muscle damage5. However, while prolonged exercise results in a reduction in the serum concentration of vitamin E, an important antioxidant, supplementation with vitamin E does not lessen exercise-induced increases in CK, nor do dogs with higher serum vitamin E concentrations before a race have a reduced likelihood of developing exertional rhabdomyolysis4–7. This suggests that oxidant injury during sustained submaximal exercise is not due to vitamin E deficiency. In addition to vitamin E and other aqueous or lipid antioxidants, resistance to an oxidant challenge is associated with the activity of antioxidant enzymes including glutathione peroxidase (GPX), superoxide dismutase (SOD) and caeruloplasmin (CER)8. The effect of competing in a long-distance sled dog race on the activity of these enzymes in red blood cells (RBCs) or plasma has not been documented. We therefore hypothesized that RBC GPX and SOD activity or plasma CER activity would decrease in dogs completing a long-distance race and vary inversely with plasma CK activity.

The objectives of this study were to measure plasma vitamin E concentration and CER activity, and RBC GPX and SOD activities, of dogs before and at the end of a long-distance sled dog race. In addition,
plasma CK and AST activities were measured as indicators of skeletal muscle damage.

Materials and methods

Blood samples for measurement of RBC SOD and GPX activities, plasma CK, AST and CER activities and vitamin E concentration were collected from Alaskan sled dogs 24 to 72 h before the start of the 1992 Yukon Quest International Sled Dog race. Additional blood samples were collected within 1 h of the end of the race from dogs sampled before the race, and from additional dogs that completed the race but from which blood samples had not been collected before the race.

Blood samples were collected before the race from 23 males and eight females of age 4.3 ± 2.0 years and weight 24.6 ± 4.0 kg, and after the race from 30 males and nine females of age 3.9 ± 1.5 years and pre-race weight 25.0 ± 2.5 kg (mean ± standard deviation). Ten of the 13 dogs that had blood drawn both before and after the race were males, three were females, and their average weight and age were 26.7 ± 0.5 kg and 4.2 ± 0.4 years, respectively. Dogs were from six teams that competed in the race. All dogs had completed 1600–3000 km in training in the four months preceding the race. Dogs sampled after the race had also collected completely 1600 km at an overall speed of 5.5 km h⁻¹, including rest stops. Ambient temperature ranged between −43 and −9°C.

Blood was collected by cephalic venepuncture into chilled evacuated glass tubes containing heparin sodium (Vacutainer; Becton Dickinson, Parsippany, NJ). Blood samples were stored on ice until processed for storage and shipping within 3 h of collection. A well-mixed sample of whole blood was retained for measurement of RBC GPX and SOD activity, and plasma for measurement of CK, AST and CER activities and vitamin E concentration, with CER collected by centrifugation (1500 g for 15 min). Samples for measurement of SOD were refrigerated before analysis. Blood for measurement of GPX activity and plasma for measurement of vitamin E and cholesterol concentrations and CER, CK and AST activities were stored frozen (−20°C). Samples were analysed within 3 days (SOD) or 1 month of collection. Plasma cholesterol concentration was measured because vitamin E is transported in blood lipids and adjustment of plasma vitamin E concentration for plasma cholesterol concentration is advantageous in assessment of vitamin E status⁹. Plasma for measurement of vitamin E was stored without air space in an air-tight container (disposable sterile cryogenic vials; Corning Glass Works, Corning, NY).

Plasma vitamin E (α-tocopherol) concentration, CER activity and RBC GPX and SOD activities were measured as previously described¹⁰–¹². Plasma CK and AST activities and cholesterol concentration were measured using an automated clinical chemistry analyzer (Coulter Electronics, Hialeah, FL).

Statistical analysis was performed to test the null hypotheses for values before and after racing for all dogs and for dogs that were examined both before and after racing. A t-test for paired data (Sigmastat; SPSS Inc., Chicago, IL) was used to test for differences among values of dogs examined twice. Values for all dogs before and after the race were compared using a t-test for independent groups. Values for CK and AST were not normally distributed and were log₁₀-transformed before analysis. Reported values are mean, or geometric mean, 95% confidence interval and range. Because of the multiple comparisons performed in the analysis, the type I error rate was set at 0.01.

Results

There were significant differences between values before and after racing for CK, AST, GPX and CER activities, and plasma albumin and total protein concentrations (Table 1). Creatine kinase and AST activities increased by approximately 300% and 170%, respectively, whereas GPX and CER activities, albumin and total protein concentrations decreased by approximately 14%, 24%, 10% and 4%, respectively, over the course of the race. The reduction in plasma CER was not significantly different to the reduction in plasma albumin concentration (P = 0.07). No change was detected for plasma vitamin E or cholesterol concentration. The concentration ratio of plasma vitamin E to cholesterol was significantly lower after racing when samples from all dogs were considered, but not when values only for dogs sampled both before and after racing were considered.

Discussion

This study has demonstrated that competing in a long-distance sled dog race results in significant declines in the activity of RBC GPX and plasma CER, two of the three major antioxidant enzymes in red blood cells or plasma. Declines in the activities of these enzymes were associated with skeletal muscle cell damage, as indicated by increased plasma activities of CK and AST. However, a causal link between decreases in antioxidant enzyme activity and exercise-induced muscle damage was not demonstrated by this study. It is not possible to determine whether reductions in antioxidant capacity contributed to muscle cell damage, whether muscle cell damage and declines in antioxidant capacity occurred simultaneously but separately, or if skeletal muscle cell damage reduced antioxidant...
Further studies are indicated to elucidate these potential explanations.

Normal oxidative metabolism involves the production of free radicals: the greater the rate of oxidative metabolism, the greater the rate of free radical generation. Exercise greatly increases oxygen consumption and hence the rate of free radical generation, and sled dogs, because of their high metabolic rate (as evidenced by their high rate of energy expenditure), may be at risk of developing exercise-induced muscle damage. Organisms accustomed to aerobic metabolism have evolved techniques for scavenging free radicals. Prominent antioxidants in mammalian systems are the glutathione peroxidase system, superoxide dismutase, caeruloplasmin and vitamin E (α-tocopherol). Glutathione peroxidase, a selenium-containing metallo-enzyme which catalyses the conversion of reduced glutathione to oxidized glutathione thereby reducing the production of peroxides, is an important scavenger of intracellular free radicals. Caeruloplasmin scavenges free radicals in extracellular fluid by catalysing the oxidation of Fe^{2+} to Fe^{3+} without release of free radicals. Vitamin E, a non-protein antioxidant, scavenges free radicals in cell membranes, during which process both the free radical and the vitamin E are consumed. Thus, representative variables of both the enzymatic (GPX, SOD, CER) and non-enzymatic (vitamin E) antioxidant systems were examined in this study.

Both vitamin E deficiency and a reduction in RBC or plasma GPX activity, and associated reductions in glutathione concentration, cause myopathy in a number of species. Vitamin E deficiency causes myopathy in dogs, although there is no association between serum vitamin E concentration and risk of developing exertional rhabdomyolysis in sled dogs. Furthermore, the lack of a difference before and after the race is surprising, given that most, but not all, studies of dogs report an exercise-induced decrease in serum vitamin E concentration. The reasons for these discordant results are not clear, but probably involve differing vitamin E intakes during racing among teams of dogs, given that exercise does not increase the rate of disappearance of deuterated vitamin E.

### Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre-race</th>
<th>Post-race</th>
<th>Pre- and post-race</th>
<th>Pre- and post-race</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK (units l^{-1})</td>
<td>153 (137–172)</td>
<td>163 (143–187)</td>
<td>661* (543–804)</td>
<td>656* (487–883)</td>
</tr>
<tr>
<td>[78–265]</td>
<td>[120–236]</td>
<td>[243–6464]</td>
<td>[277–1280]</td>
<td></td>
</tr>
<tr>
<td>AST (units l^{-1})</td>
<td>35.5 (33.1–38.1)</td>
<td>36.1 (32.8–41.4)</td>
<td>96.9* (86.2–109)</td>
<td>98.4* (81.1–121)</td>
</tr>
<tr>
<td>[12.7–30.5]</td>
<td>[25.0–57.0]</td>
<td>[50–282]</td>
<td>[50.0–158]</td>
<td></td>
</tr>
<tr>
<td>Vitamin E (μg ml^{-1})</td>
<td>18.8 (16.9–20.6)</td>
<td>15.9 (14.4–17.5)</td>
<td>16.6 (14.8–18.3)</td>
<td>17.6 (13.9–20.7)</td>
</tr>
<tr>
<td>[12.7–30.5]</td>
<td>[25.0–56.7]</td>
<td>[9.1–33.5]</td>
<td>[12.7–33.5]</td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mg dl^{-1})</td>
<td>221 (200–242)</td>
<td>204 (176–234)</td>
<td>220 (207–233)</td>
<td>224 (193–249)</td>
</tr>
<tr>
<td>[158–368]</td>
<td>[158–308]</td>
<td>[146–354]</td>
<td>[161–354]</td>
<td></td>
</tr>
<tr>
<td>Vitamin E: cholesterol (μg mg^{-1})</td>
<td>8.7 (8.1–9.3)</td>
<td>7.7 (6.3–9.1)</td>
<td>7.5* (6.9–8.1)</td>
<td>7.7 (6.9–8.5)</td>
</tr>
<tr>
<td>[4.9–11.6]</td>
<td>[4.9–11.6]</td>
<td>[4.7–12.3]</td>
<td>[3.3–10.4]</td>
<td></td>
</tr>
<tr>
<td>GPX (units g^{-1} Hb)</td>
<td>67.2 (64.1–70.3)</td>
<td>66.1 (63.5–72.7)</td>
<td>58.0* (55.7–60.3)</td>
<td>56.7* (54.3–60.5)</td>
</tr>
<tr>
<td>[44.9–82.5]</td>
<td>[47.4–82.5]</td>
<td>[41.8–78.2]</td>
<td>[48.5–66.0]</td>
<td></td>
</tr>
<tr>
<td>SOD (units mg^{-1} Hb)</td>
<td>36.9 (30.8–43.0)</td>
<td>38.7 (28.5–49.1)</td>
<td>34.7 (28.5–40.8)</td>
<td>30.6 (22.4–35.9)</td>
</tr>
<tr>
<td>[18.7–85.8]</td>
<td>[19.2–80.0]</td>
<td>[11.4–113]</td>
<td>[11.4–52.5]</td>
<td></td>
</tr>
<tr>
<td>CER (units l^{-1})</td>
<td>66 (60–72)</td>
<td>63 (54–72)</td>
<td>49* (45–54)</td>
<td>46* (40–52)</td>
</tr>
<tr>
<td>[38–95]</td>
<td>[38–92]</td>
<td>[27–82]</td>
<td>[32–64]</td>
<td></td>
</tr>
<tr>
<td>Total protein (g l^{-1})</td>
<td>59 (58–60)</td>
<td>57 (55–59)</td>
<td>53* (51–55)</td>
<td>55 (53–57)</td>
</tr>
<tr>
<td>[49–68]</td>
<td>[49–60]</td>
<td>[45–60]</td>
<td>[49–60]</td>
<td></td>
</tr>
<tr>
<td>Albumin (g l^{-1})</td>
<td>30 (29–31)</td>
<td>31 (30–32)</td>
<td>27* (26–28)</td>
<td>28* (27–29)</td>
</tr>
<tr>
<td>[27–36]</td>
<td>[29–36]</td>
<td>[45–60]</td>
<td>[26–32]</td>
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</tbody>
</table>

Hb – haemoglobin; * Geometric mean; † P < 0.01 compared with corresponding pre-race value.
from the blood of sled dogs \(^{17}\). The plasma concentrations of vitamin E detected in the current study were similar to those previously reported for dogs that had not been supplemented with vitamin E \(^{4}\).

The decline in RBC GPX activity over the course of the race is a novel finding and suggests a reduction in enzymatic antioxidant activity in red blood cells. Contrary to the present findings, another study did not detect a reduction in RBC GPX in dogs running 58 km on each of three consecutive days \(^{5}\). Moreover, exercise conditioning of Beagle dogs is associated with increases in skeletal muscle GPX activity \(^{18}\). The differing results of these studies may be a reflection of the distances run (174 vs. 1600 km), duration of the studies (3 vs. 11 days) and substrate examined (blood vs. muscle). The mechanism underlying the reduction in GPX in dogs of the current study is unknown. While in many species GPX activity closely matches serum selenium concentration \(^{19}\), it is unlikely that a decline in selenium status of the dogs during the race was the mechanism underlying the reduction in GPX. GPX in RBCs is a result of enzyme formed during erythropoiesis and thus changes in GPX activity in blood occur only as red cells are replaced by cells with different GPX activity \(^{19}\). Given that the life of canine RBCs is over 100 days, the 11 days of the race would not have been sufficient time for changes in selenium status to have produced changes in RBC GPX activity. Furthermore, withholding feed for 7-21 days results in significant increases in RBC GPX activity in Beagles, demonstrating that complete cessation of selenium intake does not, over a period of time similar to that of the current study, result in reductions in GPX activity \(^{18}\). While it is unlikely that there were changes in the amount of GPX in RBCs over the course of the study, there were changes in GPX activity.

The reductions in plasma albumin and protein concentration observed in this study are suggestive of changes in plasma volume. However, changes in plasma volume, and any consequent changes in RBC water content, would not have caused the observed change in GPX. Changes in RBC hydration would have produced identical changes in haemoglobin and GPX without a change in the ratio of the two. Thus, the observed change in GPX represents a change in its activity in RBCs, although the reason for this change is unknown.

The mechanism underlying the reduction in CER activity is unknown. Caeruloplasmin is an acute-phase protein and it would not have been surprising had its activity in plasma increased during exercise in response to muscle damage and associated inflammation. Caeruloplasmin is a copper-containing enzyme but, because serum copper concentrations were not measured, it is not known if the reduction in CER was attributable to a decline in copper status \(^{15}\). Part of the decline in CER is attributable to an overall reduction in plasma protein concentration. The reduction in plasma protein and albumin concentration, which was not significantly different to the percentage decline in CER, may be attributable to plasma volume expansion associated with prolonged and repetitive exercise, loss of protein from the vascular space, or a combination of these factors.

The results of this study suggest a reduction in enzymatic antioxidant activity in the blood of dogs participating in a long-distance sled dog race. Whether these changes are a result of reductions in the amount of enzyme protein or activity per unit mass is unknown but warrants further study. Changes in the amount of enzyme protein may be related to increased degradation of enzyme protein, decreases in the rate of production or a combination of both, while changes in enzyme activity per unit of protein may be a result of physicochemical or other factors that reduce enzyme activity. Interventions to ameliorate the decrease in activity will depend on the mechanism of the reduction in activity.

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**References**


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