Exercise stress, intestinal permeability and gastric ulceration in racing Alaskan sled dogs

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Abstract
Sustained strenuous exercise has been shown to produce gastrointestinal disease in athletic species, but the causative factors remain unknown. Since exercise results in oxidative stress and hypercortisolaemia, we tested the hypothesis that oxidative and physiological stress cause gastrointestinal disease in racing Alaskan sled dogs. Dogs from three racing teams were examined before (pre) and immediately after (post) completing a 1770-km sled dog race in approximately 11 days. Serum cortisol and isoprostane concentrations were compared with markers of gastrointestinal barrier integrity and endoscopic evidence of gastric ulceration. Gastric barrier integrity was assessed by measuring the urinary recovery of sucrose and intestinal barrier integrity was assessed using the urinary recovery ratio of lactulose to rhamnose (L/R), administered together by orogastric gavage. Exercise produced a significant increase in median serum cortisol (pre: 1040, 717–2946 pg ml⁻¹ (range); post: 8072, 2228–29 571 pg ml⁻¹; P, 0.0001) and L/R recovery ratio (pre: 0.110, 0.060–0.270; post: 0.165, 0.080–0.240; P, 0.009) but not isoprostane (pre: 1007, 656–2305 pg ml⁻¹; post: 1164, 23–4710 pg ml⁻¹; P, 0.194) concentration. The increased serum cortisol concentration was not correlated with a change in L/R recovery ratio or urine sucrose concentration. Dogs with abnormal gastric endoscopy results (61% of finishers) had higher serum cortisol concentrations than dogs with normal endoscopy results (P, 0.0007). We have demonstrated concurrent hypercortisolaemia and gastrointestinal barrier dysfunction with no correlation of the two. Thus, our data do not provide support for the hypothesis that increased serum cortisol concentration causes exercise-induced gastrointestinal disease.

Keywords: gastric ulcer; intestinal permeability; exercise; cortisol

Introduction
Strenuous exercise has been shown to lead to gastrointestinal dysfunction in humans but in 1998 Gil et al. concluded that ‘the aetiology and pathophysiology are far from clear’. Pals et al. demonstrated that exercise-induced increases in intestinal permeability are correlated with the intensity of exercise, i.e. the more strenuous the exercise, the greater the permeability of the intestinal mucosa. Participants in that study did not report any symptoms of gastrointestinal dysfunction, but it is important to note that exercise challenges in the study lasted for only 1 h. In contrast, symptoms of gastrointestinal dysfunction are quite prevalent in participants of more extended sessions of strenuous exercise such as marathons. Various hypotheses have been advanced to explain the development of exercise-induced gastrointestinal dysfunction, including visceral ischaemia and mechanical trauma, but none has been proved conclusively.

In humans, horses and dogs, strenuous exercise is associated with oxidative stress as well as physiological stress, and it is possible that one or both of these factors contribute(s) to exercise-induced gastrointestinal disease. Stress-induced increases in endogenous
glucocorticoids as well as exogenously administered glu
cocorticoids have been shown to produce gastrointesti
nal mucosal dysfunction in laboratory rodents and
gastric ulceration in dogs. Oxidative stress causes al		
teration or damage to cellular lipids, proteins and
nucleic acids, which can ultimately lead to inflammatory
damage and/or apoptosis. 8-Isoprostaglandin F2α
(isoprostane) is a marker of peroxidative lipid
damage, and has been shown to increase during repeti
tive endurance exercise in sled dogs. The concurrent
development of hypercortisolaemia, oxidative stress
and gastrointestinal disease during sustained strenuous
exercise presents the possibility of cortisol and oxidative
damage causing the gastrointestinal effects. However,
this relationship has not been examined.

Racing sled dogs are ideal animal models for exer-
cise-induced gastrointestinal disease. Distance-racing
sled dogs exercise at a sustained moderate rate for
days to weeks. Sled dogs have also been shown to
develop gastrointestinal mucosal disease as a result of
strenuous exercise. The typical sled dog team con-
ists of a relatively homogeneous population of dogs
ranging from 3 to 7 years in age and from 20 to
25 kg in body weight. Also, sled dogs exercise as
tams, ensuring uniformity of the exercise conditions
and challenge. Therefore, we chose to use racing
sled dogs to test our hypothesis that the combination
of exercise-induced physiological and oxidative stress
causes gastric ulcers and increases gastrointestinal
permeability. This hypothesis was addressed using
two specific aims. First, to determine the effects of
sustained strenuous exercise on oxidative stress, phys-
iological stress, endoscopic gastric lesions and gastric
and intestinal permeability; and second, to correlate
the measures of physiological and oxidative stress
with indices of gastrointestinal mucosal disruption.

Experimental design
This study was conducted during the 2003 Iditarod
Sled Dog Race. The Iditarod is conducted every
March, and teams of dogs cover the 1770-km course
in approximately 10–12 days. All procedures were
approved by the Oklahoma State University Insti
tutional Animal Care and Use Committee, and
informed consent was obtained from the owners of
the dogs prior to enrolling the dogs into the study.
Dogs from three teams were examined approximately
1 week before the start of the race and 24 h after com-
pleting the race. Dogs were fasted for 8–9 h prior to
examination. Blood samples were obtained at the
beginning of the examination for measurement of serum
cortisol and isoprostane concentrations. Each
dog received an isotonic solution containing sucrose,
lactulose and rhamnose by orogastric gavage. A
voided urine sample was obtained 4–6 h after receiv-
ing the sugar solution for measurement of urine
concentrations of exogenously administered sugars.
Dogs were then anaesthetized using intravenous pro-
pofol (7 mg kg−1) and gastroscopy was performed.
The endoscopic appearance of the gastric mucosa
was scored as follows:

- 0 = no lesions seen
- 1 = fewer than six areas of submucosal discolor-
ation (erythema), but no visible breaks in the gas-
tric mucosa (no bleeding)
- 2 = six or more areas of submucosal discoloration
  or a single area of disrupted (bleeding) mucosa
- 3 = more than one area of disrupted (bleeding)
  mucosa.

A urine sample was obtained during anaesthesia
(either by cystocentesis or catheterization) if a
voided sample was not available immediately before
induction of anaesthesia.

Materials and methods

Animals
Mongrel racing Alaskan sled dogs from three different
kennels were enrolled in the study. Fifty-three dogs
were examined before the race. Of these, 44 dogs
(16 for each of two teams and 12 from the third
team) started the race. All dogs were considered to
be at peak training level and not exercised within
48 h of the pre-race examination.

Sample assays
Serum cortisol and isoprostane concentrations were
measured using commercially available enzyme-linked
immunosorbent assay kits according to the manufac-
turer’s instructions (Assay Designs Inc., Ann Arbor,
MI and Cayman Chemical Co., Ann Arbor, MI, respect-
ively). Urinary sucrose, lactulose and rhamnose concen-
trations were measured using a previously
published high-performance liquid chromatography
method. Briefly, urine samples were filtered through
0.4 μm pore-size syringe filters and diluted 1:100 with
filtered, deionized water containing 0.1 g l−1 NaNO3. Se-
paration was achieved using a NaOH gradient at
1 ml min−1 flow through an anion exchange column.
Sugars were quantified by pulsed amperometric detec-
tion. The concentration of lactulose was divided by
the rhamnose concentration in the same sample for
determination of the L/R recovery ratio.

Statistics
Our first specific aim (the effect of exercise on serum
cortisol and isoprostane concentrations, urine sucrose
concentration and urine L/R recovery ratio) was evalu-
ated using the Wilcoxon signed rank test for paired
data using post-exercise samples and the corresponding pre-race samples.

For our second specific aim, Spearman regression was used on pre-race and post-race samples to determine the correlation between serum cortisol or isoprostane concentrations and gastric or intestinal permeability indices. Also, data were pooled into two groups according to corresponding gastric ulcer scores, normal (gastric ulcer score = 0, n = 60) or abnormal (gastric ulcer score > 0, n = 11), because of the small number of dogs receiving a non-zero ulcer score. A Mann–Whitney test was then used to compare serum cortisol and isoprostane, and urine sucrose concentrations that were grouped according to a normal or abnormal gastric ulcer score. Data were analysed using GraphPad Prism 4 software (GraphPad Software, Inc., San Diego, CA). Data are presented as median with 25th and 75th interquartiles and range in the figs. and as median with range in the text. A P-value of <0.05 was considered statistically significant.

**Results**

Eighteen of the 44 dogs completed the race: nine on each of two teams. These two teams finished the race in approximately 11 days. The remaining team was withdrawn from the race. However, two dogs in the finishing teams were not examined pre-race and therefore could not be included in the paired analysis comparing pre-race and post-race. Therefore, data analysis addressing the first specific aim was conducted with n = 16 (only dogs that were examined both pre-race and post-race).

Median post-race serum cortisol concentration (8072, 2228–29 571 pg ml\(^{-1}\)) was significantly greater than median pre-race serum cortisol concentration (1040, 717–2946 pg ml\(^{-1}\)) (Fig. 1a; \(P < 0.0001, n = 16\)). In contrast, there was no significant effect of exercise on median serum isoprostane concentration from pre (1007, 656–2305 pg ml\(^{-1}\)) to post (1164, 23–4710 pg ml\(^{-1}\)) (Fig. 1b; \(P = 0.194, n = 16\)). The median urinary L/R recovery ratio increased significantly after exercise from pre (0.110, 0.060–0.270) to post (0.165, 0.080–0.240) (Fig. 1c; \(P = 0.009, n = 16\)). In contrast, the median urinary sucrose concentration was not significantly increased after exercise from pre (1.50, 0.00–10.90 mg ml\(^{-1}\)) to post (0.00, 0.00–4.30 mg ml\(^{-1}\)) (Fig. 1d; \(P = 0.095, n = 16\)).

All data from all dogs (both pre-race and post-race) were pooled to determine whether significant correlations existed between markers of stress and gastrointestinal mucosal dysfunction. Although 53 dogs were examined pre-race and 18 dogs post-race (total \(n = 71\)), urine could not be obtained from seven dogs and serum from two dogs was lost. Thus, the full number of dogs could not be included in each analysis and the number used is listed with the corresponding analysis. There was no correlation between serum cortisol concentrations and urine sucrose concentrations.
(Spearman’s \( r = -0.2, P = 0.11, n = 64 \)) or urinary \( L/R \) recovery ratios (Spearman’s \( r = 0.18, P = 0.15, n = 64 \)). Serum isoprostane concentrations were weakly correlated with urine sucrose concentrations (Spearman’s \( r = 0.37, P = 0.0028, n = 64 \)) and urine \( L/R \) (Spearman’s \( r = 0.27, P = 0.03, n = 64 \)). Dogs with abnormal gastric endoscopy findings (11 of 18; 61% of examined finishers) had a significantly higher median serum cortisol concentration (1717, 605–21 620 pg ml\(^{-1}\)) compared with dogs with normal gastric endoscopy (5056, 2228–29 571 pg ml\(^{-1}\)) (Fig. 2a; \( P = 0.0007, n = 69 \)). Dogs with normal gastric endoscopic findings did not differ in median serum isoprostane (999, 37–2942 pg ml\(^{-1}\)) or urinary sucrose (0.55, 0.00–2.10 m\( \mu \)gm l\(^{-1}\)) concentrations from those with abnormal gastric endoscopic findings (serum isoprostane: 1118, 23–4710 pg ml\(^{-1}\); urinary sucrose: 0.00, 0.00–4.30 \( \mu \)g ml\(^{-1}\)) (Fig. 2b and 2c, respectively) \( (P = 0.43, n = 69 \) and \( P = 0.20, n = 64 \), respectively).

**Discussion**

**Cause of increased cortisol**

The increased release of glucocorticoids in response to exercise is complex and conclusions regarding this response have been variable. It has been demonstrated that cortisol is elevated after exhaustive exercise in dogs\(^{15,16}\), horses\(^{12–14}\) and humans\(^{11}\). Also, in the present study we have demonstrated an increase in serum cortisol concentration in dogs sustaining exercise for an extended period of time. These dogs completed the 1770 km in an average of 11 days. This is presumably sufficient stress to cause the demonstrated increased release of cortisol. Other potential stressors contributing to this response could also be gastric mucosal damage (discussed below) and the increased demand for metabolic substrates due to the sustained energy requirement coupled with decreased caloric intake relative to this need. Increases in serum glucocorticoid concentrations have been noted in rats undergoing caloric restriction\(^{27}\). However, even with total energy expenditure estimated at 47 000 kJ day\(^{-1}\) in racing sled dogs, they are fed to appetite and estimates of digestible energy intake approximate those of energy expenditure\(^{28}\). The withholding of food for 8 h prior to serum collection may exacerbate any possible caloric restriction, but withholding food was done prior to both pre- and post-exercise measurements to maintain study symmetry. Thus, caloric restriction is not likely a large contributing stressor leading to the demonstrated increase in serum cortisol concentration after exercise.

Two other studies have examined cortisol release in response to exercise in sled dogs. Hammel *et al.*\(^{16}\) showed an increase in total plasma corticosteroids in response to an exhaustive 19-km run. However, Hinchcliff *et al.*\(^{29}\) measured serum cortisol at three points during a 1610-km race, starting at the halfway point, and did not report an increase. Their initial measurement was made after a mandatory rest for 36 h, with the following two measures within 1 h of completing a 257-km leg and a 668-km leg, respectively. Samples were taken only from dogs that were deemed fit to continue the race at each checkpoint. This finding may indicate a plateau of cortisol concentration that was not altered by the 36-h rest. Another possibility is that sampling only dogs that were fit to continue may have skewed the results. In the present study, there was an increase in serum cortisol concentration when post-exercise samples were compared with their corresponding pre-race samples. Hinchcliff *et al.*...
suggested that the dogs in their study were not exercising at an intensity sufficient to increase cortisol level, and that the dogs were accustomed to the intensity and duration of the race. However, an alternative interpretation more consistent with our results, as well as those of previous reports, is that initiation of sustained strenuous exercise causes an increase in serum cortisol concentration that persists for at least 24–36 h after cessation of the exercise.

**Effects of cortisol on gastrointestinal tract**

Glucocorticoid effects on the gastrointestinal tract are evident with exogenous dosing. However, the effects of endogenous glucocorticoid release are not well understood. High doses of exogenous glucocorticoids reliably produce gastric haemorrhage in otherwise healthy dogs. Also, the proliferation of cultured rat intestinal epithelial crypt cells is slowed in a dose-dependent manner by hydrocortisone. Glucocorticoid stress increases intestinal permeability in rats given dexamethasone and is potentiated by a lack of food in the gut. Environmental stress resulting in elevated endogenous glucocorticoids has also been shown to increase mucosal permeability and is suggested to be mediated by adrenocorticoids in rats. These findings, combined with ours of increased serum cortisol concentrations plus increased urine L/R recovery ratio and gastric ulceration, could indicate a role for glucocorticoids in exercise-induced gastrointestinal mucosal disease. Serum cortisol concentrations were increased in dogs with endoscopic gastric lesions, further supporting an association between exercise-induced hypercortisolaemia and gastrointestinal mucosal permeability. However, this association cannot distinguish between the possibilities that: high concentrations of endogenous cortisol cause gastrointestinal mucosal disease; the exercise-induced gastrointestinal mucosal disease causes elevated endogenous cortisol; or that the two findings are the independent results of a common stressor.

**Cortisol and intestinal permeability**

We demonstrated an increase in intestinal mucosal barrier permeability due to exercise in racing sled dogs that does not seem to be directly related to serum cortisol concentration. Spot measurement of urinary L/R recovery ratio has previously been validated as a measure for intestinal permeability in dogs. Increased L/R recovery ratio is assumed to indicate decreased intestinal barrier integrity by increased tight junction permeability to lactulose. Increases in L/R recovery ratio have been demonstrated in exercising humans and results seem to be directly correlated with exercise intensity. However, cultured intestinal epithelial cells can show an increase in tight junction integrity due to glucocorticoid dosing in addition to the decreased cell turnover mentioned above. Exercise-induced hypercortisolaemia and intestinal mucosal permeability failed to correlate well on a subject-by-subject basis in our study, suggesting at least that the association is not a simple cause-and-effect relationship.

**Cortisol and gastric ulcers**

Gastric ulcer score was increased in response to exercise, and median cortisol concentration was higher in dogs with gastric lesions; however, a cause-and-effect relationship was not conclusively evident. It is generally accepted that corticosteroids induce gastric ulcers; however, one study utilizing healthy dogs used large doses of exogenous synthetic glucocorticoids that are more commonly given to patients with central nervous system trauma. Thus, while previous studies suggest that corticosteroids in general may cause gastric ulceration, they do not prove that high levels of endogenous cortisol can cause the same pathology. The results of our study are unavoidably confounded by the stress that caused the increased cortisol concentrations. In fact, this stress could be the gastric ulceration itself, in effect reversing the proposed cause and effect. Gastric ulceration increases the activity of mechanosensitive vagal afferents and direct electrical vagal afferent stimulation increases activity of the hypothalamic-pituitary-adrenal axis in rats. Thus, gastric mucosal damage may sensitize vagal afferents leading to increased cortisol release in these racing sled dogs. Alternatively, the physiological stress may simply cause both increased cortisol and gastric ulceration through separate pathways. As noted by Vatistas et al., a training regimen that elicited and maintained gastric ulcers failed to cause increased cortisol levels in Thoroughbreds. Added to this uncertainty is the evidence that corticosteroids have a gastroprotective role in rats subjected to water immersion stress or to low-temperature restraint stress. The protective mechanism is suggested to be due to the maintenance of gastric microcirculation. Increased nervous stimulation caused by gastric inflammation could lead to increased cortisol release that mediates gastroprotection. Although glucocorticoids may have a gastroprotective effect during stress, our data indicate that, at the very least, these effects are insufficient to be completely protective. Our data indicate an association of abnormal gastric endoscopy and hypercortisolaemia. However, our data are insufficient to draw a mechanistic conclusion regarding the role of endogenous cortisol in exercise-induced gastric ulceration.

**Oxidative stress measure**

Previous studies have detected changes in markers of oxidative stress after exercise, with one such
study utilizing isoprostanes in sled dogs; however, we did not detect such an effect. It is likely that our failure to find such a change is based on methodological constraints rather than an absence of oxidative stress during exercise of this duration. Exercise-induced oxidative stress can be shown reliably in many exercise subjects performing at different exercise intensities and durations. Isoprostane or 8-iso-prostaglandin F2α is a non-enzymatic product of arachidonic acid peroxidation. The elimination half-life from plasma of rabbits has been found to be approximately 4 min, while the elimination half-life of its isomer, prostaglandin F2α, has been approximated at 1 min in anaesthetized dogs. The half-life of these compounds is thus likely to be short in these dogs, assuming a decrease in free radical production and subsequent peroxidation of lipids in the hours after exercise. Thus the timing of collection may have been a factor in detecting a difference in serum concentrations. Vitamin E supplementation has been initiated into many racing sled dog diets. Information on dietary supplements utilized during the race was not obtained and we cannot disregard the possibility of an increased antioxidant capacity during the race due to dietary antioxidant supplementation. Finally, the possibility of pre-existing oxidative stress in the week prior to the race must be considered. Because the dogs were not exercised within 48 h of the initial blood collection, any pre-existing oxidative stress is not likely to be due to exercise. Serum isoprostane concentrations are short-lived and the training intensities are tapered prior to the race. The isoprostane concentrations detected within the samples may also be indicative of post-collection oxidation of lipids. Experiments by Morrow et al. revealed that human serum contains necessary cofactors to allow auto-oxidation of lipids within the sample. Concentrations of isoprostane in samples frozen for several months at −20°C were approximately 50-fold higher than in fresh samples, although the kinetics at room temperature was not reported. Our results may be biased by the lack of immediate freezing of samples due to the need for post-collection processing, as they are consistent with the range of concentrations reported (1000–4000 pg ml⁻¹). Isoprostanes have been consistently used as a marker of oxidative stress, and our findings warrant a review of the timing and route of their collection and attention to prompt preservation and analysis.

In conclusion, in the present study we have shown an increase in gastric ulcers, intestinal permeability and serum cortisol concentration due to sustained strenuous exercise in racing sled dogs. Our study could not define which occurs first, and coupled with investigations revealing the protective effects of cortisol and the ability of gastric mucosal damage to increase cortisol, there is little evidence that endogenous cortisol causes gastric ulcers. Also, we did not demonstrate a correlation of intestinal permeability with cortisol. Thus, the effects of cortisol on exercise-associated gastrointestinal dysfunction do not appear to be simple, direct effects. Future experiments identifying the role of glucocorticoids will require either locally administered cortisol or its removal to identify the direct effects. Studies utilizing antioxidant supplementation are planned to further identify the role of local oxidative stress.

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References

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