Physiological effects of hunting red deer (*Cervus elaphus*)

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SUMMARY

When red deer (*Cervus elaphus*) were hunted by humans with hounds the average distance travelled was at least 19 km. This study of 64 hunted red deer provides the first empirical evidence on their state at the time of death. Blood and muscle samples obtained from hunted deer after death were compared with samples from 50 non-hunted red deer that had been cleanly shot with rifles. The effects on deer of long hunts were (i) depletion of carbohydrate resources for powering muscles, (ii) disruption of muscle tissue, and (iii) elevated secretion of β-endorphin. High concentrations of cortisol, typically associated with extreme physiological and psychological stress, were found. Damage to red blood cells occurred early in the hunts; possible mechanisms are discussed. Taken together, the evidence suggests that red deer are not well-adapted by their evolutionary or individual history to cope with the level of activity imposed on them when hunted with hounds.

1. INTRODUCTION

The debate about the welfare of red deer (*Cervus elaphus*) hunted with hounds has not been informed by much evidence. To some, the hunted deer's extreme fear and exhaustion are obvious (LACS 1992). Others suggest that, as hunting with hounds is similar to the actions of natural predators, red deer are well-adapted to this process and do not suffer unduly during the chase (Lloyd 1990). Some clues as to the likely consequences of hunting may be obtained from what is known of the biology of red deer.

Red deer evolved in temperate forest (Okarma 1995; Putman 1988) and typically move only short distances in the course of a day (Carranza *et al*. 1991; Catt & Staines 1987; Jeppesen 1987; Putman 1988). Their principal predator, the wolf (*Canis lupus*), catches them after ambushing and short chases (Nelson & Mech 1991; Okarma 1997). By contrast, hunted red deer may be followed by hounds for many hours (Bateson 1997). The issue of how the deer cope can be approached by direct examination of their state after a hunt. We studied the behaviour and physiology of deer hunted by hounds in order to provide quantitative information on how they respond (Bateson 1997). Here we present the results of the assays of blood and muscle samples taken after hunts of different distances and durations, and compare them with samples from non-hunted deer.

2. METHODS

(*a*) *Study animals*

Blood samples taken over a full year from September 1995 were obtained from red deer hunted with hounds by the Devon and Somerset Staghounds and the Quantock Staghounds. The autumn stag-hunting (adult males) season lasted from mid-August to late October, the hind-hunting (adult females) season from the beginning of November to the end of February, and the spring stag-hunting (young males) season from the beginning of March until the end of April. The hunted deer were killed with a shot to the head from a humane killer, or a shotgun fired from close range. Samples were also obtained from a non-hunted comparison group of wild red deer from south-west England and north-west Scotland. These were clean-shot in the head or upper neck with a rifle by professional stalkers. A limited number of skeletal muscles were also obtained. The numbers of samples from autumn stags, hinds, and spring stags in each group are given in table 1. Of the hunted deer that provided muscle samples, 15 also provided blood samples; and of the eight non-hunted deer, one provided a blood sample; all were animals from south-west England. Three hunted deer from which blood was obtained have not been included because no information was available about these hunts.

(*b*) *Collection of blood samples*

Free-flowing blood was collected during exsanguination into 10 ml tubes (Vacutainer, Becton Dickinson) containing lithium heparin, and 7 ml tubes containing fluoride oxalate. The blood from the hunted animals was collected as soon as possible after death by the huntsmen, and the blood from each non-hunted animal was collected by the stalker who shot the deer. Two blood samples taken from the heart or body cavity were not unusual on any measure, and were therefore also included in the analysis. Laboratories did not perform assays on seven (10.9%) of the blood samples from hunted deer, and five (10%) from non-hunted deer; it was judged that the extent of haemolysis in these samples would have affected the assays. Not all samples were chilled after collection, and time from collection of blood to centrifugation and freezing varied (median time = 4.06 h, inter-quartile ranges (IQRs) 2.88–5.69). Blood was centrifuged in an MSE Mistral 2000 or Heraeus Labofuge at 3000 r.p.m. for 5 min, and plasma fractions were removed, divided into multiple aliquots and stored frozen at 20 °C.

Table 1. *Numbers of hunted (H) and non-hunted (NH) red deer sampled for blood and muscle*

hunting season	blood H	blood NH	muscle H	muscle NH
autumn stags (Aug–Oct)	30	11	0	0
hinds (Nov–Feb)	9	30	3	4
spring stags (March–April)	22	2	16	4
total	61	43	19	8

(*c*) *Assays of blood and muscle*

Assays were performed on blood samples collected in tubes containing lithium heparin, or fluoride oxalate in the case of glucose. Plasma was kept frozen when transported to the laboratories where analyses were carried out. Here we report on 16 separate measurements. Cortisol was measured by Bloxham Laboratories Ltd (Teignmouth). β-endorphin was assayed by the Department of Animal Physiology and Nutrition, University of Leeds, UK. Assays of lactate and free fatty acids were carried out by the Department of Clinical Biochemistry at Addenbrooke's Hospital, Cambridge, UK, and of lactase dehydrogenase isoenzymes at the Department of Veterinary Clinical Medicine at the University of Cambridge. All other biochemical measures were carried out at the Department of Clinical Veterinary Sciences (Division of Companion Animals), University of Bristol, Langford, UK. Details of intra-assay and inter-assay coefficients of variation are being prepared (Bradshaw & Bateson (1998)). Samples were coded to ensure that assays were carried out blind by the laboratories. Measurements of plasma and muscle pH were not blind, having been carried out by ourselves.

Sample sizes for the groups of tests carried out by each separate laboratory vary because it was not always possible to obtain a sufficient quantity of plasma from each deer to send a sample to every laboratory. After most analyses had been started, we decided to test for bilirubin, free fatty acids, glucose, haem, lactate and isoenzymes of lactate dehydrogenase. In all these cases we had to rely on spare plasma samples. In the case of glucose, sample sizes are lower than for many variables because blood collected in tubes containing lithium heparin was unsuitable.

Collection of samples in the field meant that it was impossible to standardize the time from death to centrifugation of the blood. In order to provide some guidance as to the lability of the measures, a study was conducted on 17 farmed deer in which the time from blood collection to centrifugation was varied. Four variables were significantly affected. The values for these variables were corrected using the gradients obtained from the time-course study. The mean gradients and 95% confidence limits were as follows: free fatty acids, $0.97\,\mathrm{mmol\,l^{-1}\,h^{-1}}$ (0.71–1.23); lactate, $0.05\,\mathrm{mmol\,l^{-1}\,h^{-1}}$ (0.03–0.06); potassium, $0.24\,\mathrm{mmol\,l^{-1}\,h^{-1}}$ (0.15–0.33); and sodium, $-0.21\,\mathrm{mmol\,l^{-1}\,h^{-1}}$ (-0.35 to -0.07). We do not know whether any of these changes would have been greater in wild deer that had been hunted; if they had been greater, they would have added to the variance in the data.

Cortisol was determined by chemiluminescent enzyme immunoassay using an Immulite automated analyser (Diagnostic Products, LA); β-endorphin by radioimmunoassay using the method of Leshin & Malven (1984); lactate, and free fatty acids by enzyme-linked reactions using a centrifugal analyser (Monarch, Instrumentation Laboratory); and haem and total bilirubin by commercial non-automated colorimetric methods (Sigma Diagnostics and Reagents Applications, respectively). Electrolytes were assayed at room temperature using a Nova 14 CRT (Nova Medical) or Kone Microlyte 3+2 analyser; and all other metabolites and enzymes were assayed at 37 °C using a Kone Supra Discrete auto-analyser (Kone Instruments) except for the LDH isoenzymes, which were determined electrophoretically (Broom *et al*. 1996). Plasma acidity was measured using a combination pH electrode (Russell pH Ltd) and a portable microcomputer pH meter (HI 8424, Hanna Instruments). Glycogen remaining in muscle at the time of death was assessed indirectly by measuring muscle pH 24 h after death, since anaerobic respiration within the cells converts all remaining glycogen to lactic acid (Warriss *et al*. 1989). Muscle samples, collected post-mortem, were incubated in self-seal plastic bags at room temperature for 24 h, at the end of which approximately $2.0 \times 2.0 \times 0.5$ cm of muscle was homogenized with 5 ml of deionized water, centrifuged and the supernatant acidity measured. The higher the pH, the lower the inferred level of glycogen at the time of death.

(*d*) *Extent of hunts*

Accounts of hunts, prepared by people with long experience of hunting, are published in the *West Somerset Free Press*. A grid reference was found for each place mentioned in the published report. The straight-line distances between each grid point were calculated with a simple computer program, and the total distances travelled accumulated. Start and end times of hunts were obtained from our own field notes or estimated from the published account; these provided an estimate of hunt duration. The distance measure was significantly correlated with the duration measure, but by no means perfectly ($F_{1,62}=20.7$, $p<0.001$). Distance was likely to be underestimated considerably because straight-line measurements were used. To minimize errors, both distance and duration measures were standardized to normal distributions with means of zero and standard deviations of 1.00. These were averaged to provide a composite standardized measure ('extent of hunt'). Three was added to each score so that all scores were positive.

(*e*) *Statistics*

The General Linear Model on the Minitab Release 10.51 Xtra package was used for all statistical analyses. The distribution of residuals was examined for each measure. When the residuals were non-random, the data were transformed by taking natural logarithms. In such cases, population data are given as medians with IQRs. F values for the comparison between hunted and non-hunted deer are reported when time of year was entered as a covariate even when time of year was not itself a significant source of variation. For correlations within the hunted group the F value is reported for the extent of the hunt entered as a covariate when the main factor was the hunting season. Once again, this was done even when the hunting season was not a significant source of variation. The data from the hunted animals were ranked according to

Table 2. *Results of plasma assays for non-hunted and hunted deer, and to deer hunted to different extents*

		non-hunted		hunted		hunt extent			
variable	unit	mean (median)	s.d. (IQR)	Mean (median)	s.d. (IQR)	0.87–2.32	2.33–2.94	3.02–3.63	3.76–4.63
glucose	$mmol\,l^{-1}$	6.1	3.0	6.9	5.2	12.6	8.7	4.9	3.4
lactate[a]	$mmol\,l^{-1}$	9.2	3.3	12.2	9.2	23.7	15.4	5.9	6.0
acidity	pH	8.05	0.18	7.95	0.25	7.68	7.88	8.09	8.16
FFA[a,b]	$mmol\,l^{-1}$	(69.5)	(39.6–128)	(833)	(460–1168)	406.9	722.0	1053	1079
CK[b]	$u\,l^{-1}$	(270)	(203–320)	(3459)	(1791–5921)	1468	3382	3576	6003
AST[b]	$u\,l^{-1}$	(61.5)	(49.0–83.0)	(177)	(131–291)	116.0	150.0	194.0	295.5
LDH[b]	$u\,l^{-1}$	(806)	(669–907)	(1732)	(1187–2537)	1257	1295	1829	2711
LDH5	%	3.3	6.0	27.8	11.8	13.6	24.2	27.1	36.1
cortisol[b]	$nmol\,l^{-1}$	(<2.7)	(<2.7–14.4)	(197)	(161–236)	147.0	211.0	225.0	192.5
β-end	$pmol\,l^{-1}$	132.0	74.2	280.8	168.3	175.7	277.9	308.8	359.2
haem[b]	$\mu mol\,l^{-1}$	(3.2)	(1.7–4.7)	(32.9)	(20.6–68.2)	24.6	47.5	63.0	35.4
bilirubin[b]	$\mu mol\,l^{-1}$	(5.0)	(4.4–5.9)	(14.1)	(11.0–20.4)	9.0	17.1	14.6	17.4
sodium[a]	$mmol\,l^{-1}$	138.4	4.6	145.8	5.2	145.9	144.2	146.4	146.5
potassium[a]	$mmol\,l^{-1}$	8.0	3.1	6.2	2.7	7.1	5.7	5.7	6.5
chloride	$mmol\,l^{-1}$	97.4	6.0	100.1	4.4	91.1	99.3	100.2	102.4
creatinine[b]	$\mu mol\,l^{-1}$	(117)	(107–128)	(167)	(131–207)	154.0	199.0	138.0	167.0

[a]The concentrations of these variables were labile, so correction factors were applied for the time between killing and centrifugation.
[b]These measures were not normally distributed so medians and inter-quartile ranges (IQRs) are given instead of means. Medians and IQRs are given in brackets for the non-hunted deer and for the complete group of hunted deer.

the extent of the hunt and divided into four nearly equal groups, three consisting of 15 animals, and the most extensively hunted group consisting of 16 animals.

3. RESULTS

The mean distance travelled by 64 hunted deer from which blood and/or muscle samples were obtained was 19.10 km (s.d. = 7.39) and the mean duration of the hunts was 3.12 h (s.d. = 1.21). An overall calculation of speed would be misleading because of the likely underestimate of actual distance travelled and the episodic nature of the hunts. The behaviour of the hunted deer is characterized by short periods of intense exertion interspersed with periods of hiding or relative inactivity (Bateson 1997). Distance and duration were combined to produce a composite measure called extent of the hunt (see §2). The means (and s.d.) in standardized units for the three hunting seasons are as follows: autumn stags = 2.75 (0.84), hinds = 2.77 (0.68), spring stags = 3.44 (0.81). The overall heterogeneity is statistically significant ($F_{2,58} = 5.0$, $p = 0.01$) with the spring stags being hunted more extensively than the autumn stags or hinds.

Means (or medians when the data are not normally distributed) for 16 measures for non-hunted and hunted deer are given in table 2. Standard deviations (or IQRs) are also given. Sample sizes and the results of the between-group and within-hunted group statistical tests are given in table 3. In each case the procedure has been conservative, because time of year (or hunting season) was entered in the analyses even when it was not a significant source of variation. Some measures were affected by season, as may be seen in table 3.

Since animals from the three seasons were unequally distributed in both hunted and non-hunted groups, comparisons were repeated using only stags hunted in the autumn. All the differences that were highly significant remained so except for β-endorphin.

Glucose, lactate, acidity and free fatty acid (FFA) concentrations are shown in figure 1 and table 2. It should be noted that, because the samples were exposed to air, pH was consistently higher than would normally be expected in mammalian plasma. Glucose and lactate concentrations and acidity in hunted deer differed systematically from those for non-hunted deer. In deer hunted to the least extent, glucose concentrations were significantly higher than in non-hunted animals ($F_{1,16} = 8.4$, $p < 0.05$), and in those hunted to the greatest extent they were significantly lower ($F_{1,18} = 9.6$, $p < 0.01$). The pattern was the same for lactate ($F_{1,46} = 84.2$, $p < 0.001$; $F_{1,47} = 8.2$, $p < 0.01$) and the opposite for pH ($F_{1,55} = 40.2$, $p < 0.001$; $F_{1,56} = 4.3$, $p < 0.05$).

Results of measuring acidity in four different muscles 24 h after death are shown in figure 2. In muscles from undisturbed animals, the pH was low because glycogen remained to be converted to lactic acid. The pH was high in deer hunted for more than the average extent of hunting, suggesting a depletion of muscle glycogen.

The activities of three enzymes associated with striated muscles, creatine kinase (CK), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) increased in the plasma with the extent of the hunt, and were significantly higher in hunted than in non-hunted deer (see table 3). The isoenzyme of LDH

Table 3. *Sample sizes, standard deviations (or inter-quartile ranges), and the results of the between-group and within-hunted group statistical tests*

(In each case, the first degree of freedom (d.f.) is 1. In comparisons between hunted and non-hunted deer, the second d.f. is N (non-hunted)+N (hunted) − 3. In regressions within the hunted group the second d.f. is N (hunted) − 4.)

variable	non-hunted N	hunted N	hunted vs non-hunted F	within hunted F	season F
glucose[c]	7	36	0.5	54.8***	3.7*
lactate[a,c]	36	43	7.9**	38.4***	0.5
acidity[c]	42	58	6.6*	50.0***	2.7
FFA[a,b]	36	43	104.0***	39.5***	7.2**
CK[b]	40	54	170.3***	16.2***	0.5
AST[b]	40	54	77.5***	19.1***	2.4
LDH[a,b]	40	53	88.5***	10.9***	3.1
LDH5	34	33	62.6***	10.8**	3.5*
cortisol[b]	42	60	554.5***	11.6***	4.5*
β-endorphin	41	58	20.2***	4.6*	0.6
haem[b]	40	52	202.0***	3.4	2.5
bilirubin[b]	39	49	166.8***	33.8***	13.4***
sodium[a]	40	53	44.4***	0.6	3.2*
potassium[a]	40	53	7.6**	0.3	0.0
chloride[c]	40	44	2.1	9.3**	1.1
creatinine[b]	40	53	61.2***	0.8	12.1***

*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.
[a]The concentrations of these variables were labile, so correction factors were applied for the time between killing and centrifugation.
[b]These measures were not normally distributed, so inter-quartile ranges are given instead of standard deviations, and statistical tests were applied to natural logarithmic transformations. The General Linear Model was used for each statistical test.
[c]The values for the hunted animals are different from those for non-hunted animals in hunts of the shortest and longest extents.

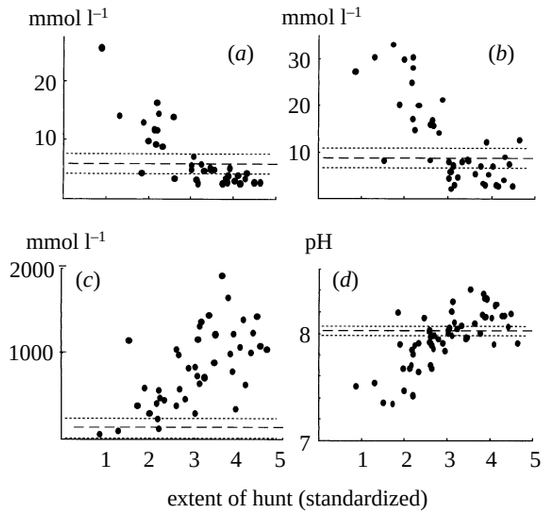

Figure 1. The values of (*a*) glucose, (*b*) lactate, (*c*) free fatty acids, and (*d*) acidity for each red deer in the hunted group plotted against the extent of the hunt. Extent is a composite of standardized distances and durations of the hunts. The mean values for the non-hunted group are shown as dashed lines and the 95% confidence limits as dotted lines.

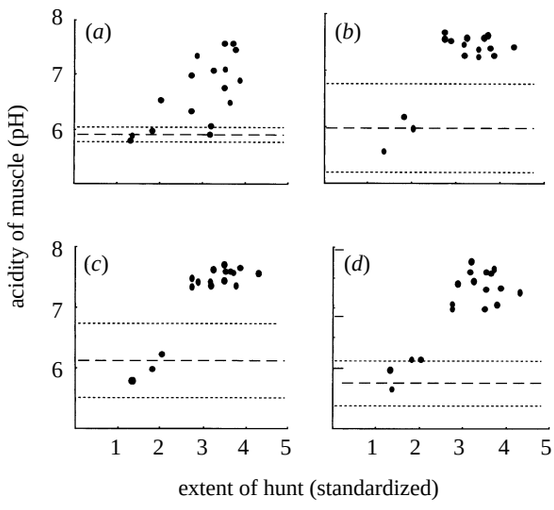

Figure 2. The acidity, measured 24 hours after death, of four muscles from hunted red deer plotted against the extent of the hunt. Extent is a composite of standardized distances and durations of the hunts. The mean values for the non-hunted deer are shown as dashed lines and the 95% confidence limits as dotted lines. (*a*) The *sternocephalic* is from the neck, (*b*) the *longissimus dorsi* from the trunk, (*c*) the *supraspinatus* from a forelimb, and (*d*) the *psoas* is from the loin.

specifically associated with skeletal muscle, LDH5, showed an identical pattern. The concentration of this isoenzyme as a percentage of all five isoenzymes of lactate dehydrogenase was significantly higher in the plasma of hunted deer, and was correlated with the extent of the hunt.

In most undisturbed animals cortisol concentrations were below detectable limits but in hunted deer the proportional increase was very great. β-endorphin concentrations in deer hunted to the least extent were not much higher than non-hunted deer, but then increased with more extensive hunts. β-endorphin was also correlated with the residuals of the regression

between CK and the extent of the hunt ($F_{1,50}=10.8$, $p<0.01$).

Haem concentrations were more than ten times higher in the hunted animal than in the non-hunted animals. Time from death to centrifugation was slightly, but significantly, longer in the non-hunted than in the hunted deer ($F_{1,102}=5.8$, $p<0.05$), but did not account for the variation in haem when entered as a covariate in the statistical analysis ($F_{1,89}=0.9$). Uncorrected concentrations of potassium were not correlated with haem in hunted deer ($F_{1,50}=0.1$) and positively correlated in non-hunted deer ($F_{1,38}=4.3$, $p<0.05$). When haem and the extent of the hunt are both entered into a multiple regression with bilirubin, both account for significant proportions of the variance (respectively, $F_{1,46}=23.3$, $p<0.001$; $F_{1,46}=12.1$, $p=0.001$). Similarly, when haem and the extent of the hunt are both entered into a multiple regression with CK, both account for significant proportions of the variance (respectively, $F_{1,49}=8.0$, $p<0.01$; $F_{1,49}=25.9$, $p<0.001$).

Since haem might have affected the reactivity of other assays, it was entered as a covariate in the comparison between hunted and non-hunted groups or into the multiple regression when examining the association between the extent of the hunt and other variables. Only one conclusion was affected: this was the association between the extent of the hunt and the concentration of cortisol. This association should, therefore, be treated with caution.

Creatinine and sodium concentrations were significantly higher in hunted than in non-hunted deer. Neither of these changed with the extent of the hunt. The data suggest that, as in normally exercised animals, the blood became more concentrated at the onset of the chase. Since the effect of hunting was to lead to an immediate concentration of the blood, statistical comparisons between hunted and non-hunted deer were repeated after each value had been divided by the value for creatinine. No alterations in the pattern of the data were found, and all differences that were highly significant before the correction remained so afterwards.

In some cases, several assays were required to analyse all samples. The batch number of the assay was entered as a covariate into the comparison between hunted and non-hunted groups or into the multiple regression when examining the association between the extent of the hunt and other variables. Differences between assays generally had no effect, but accounted for a significant amount of variation in LDH both in analyses between groups ($F_{1,90}=27.7$, $p<0.001$) and that within the hunted group ($F_{1,49}=17.4$, $p<0.001$). However, the difference between the groups and the association between enzyme and the extent of the hunt remained highly significant.

Of a number of other relatively small changes and differences, attention is drawn to the concentrations of chloride in hunted deer, which rose from the same concentration as that of non-hunted deer to significantly higher concentrations when the most extensively hunted quartile was considered. Sodium was significantly higher in hunted deer (see table 3) irrespective of the extent of the hunt. When compared with non-hunted animals, the ratio of sodium to chloride was significantly higher in the least-hunted animals ($F_{1,46}=9.1$, $p<0.01$), but was not different in the animals hunted to the greatest extent ($F_{1,51}=0.3$).

4. DISCUSSION

In the least extensive hunts, the plasma glucose and lactate concentrations were above those for non-hunted animals, as would be expected in short bouts of normal exercise (Brooks *et al.* 1996). The evidence for extreme depletion of carbohydrate resources for powering muscles is strong in more extensive hunts. After death, the muscle cells convert all remaining glycogen to lactic acid and pH falls, the final lactate concentration being reached after approximately 24 h (Warriss *et al.* 1989). In muscles used for locomotion the pH reached an asymptote, indicating total depletion of glycogen. The sternocephalic muscle is not used in running, and depletion occurred more slowly here. However, it is striking that in the most extensive hunts even in this muscle the pH 24 h after death is the same as in the other muscles. FFA concentrations at death were strongly positively correlated with the extent of the hunt. The fatty acids were mobilized as carbohydrate became depleted. While this is unsurprising, it would have been accompanied by a significant reduction in the capacity for work (Brooks *et al.* 1996). Taken together, the evidence suggests that in at least a quarter of the hunts leading to a kill, deer had minimal carbohydrate resources with which to power their muscles. Their blood sugar was significantly lower than that of undisturbed animals. This conclusion is consistent with behavioural observations of their state of exhaustion (Bateson 1997).

The hunted deer showed considerable signs of muscle disruption. After more extensive hunts, the activities of CK, AST, LDH and its isoenzyme specific to skeletal muscle (Goddard *et al.* 1997), were very considerably higher than in the non-hunted animals. In studies of horses, a CK concentration greater than 10 000 units l^{-1} is taken as indicative of myopathy (Volfinger *et al.* 1994). In our sample of 61 red deer, four (6.6%) had concentrations greater than 10 000 units l^{-1} and ten (16.3%) had concentrations greater than 7500 units l^{-1}. After muscle damage, muscle enzymes continue to leak into the blood for some hours, and so concentration would have continued to rise in animals that were not killed at the end of a hunt. 'Capture myopathy' found in wild animals after a long chase or much struggling at the time of capture has been known for many years in a number of species, including red deer (Jarrett *et al.* 1964; McAllum 1985). This syndrome is not fully understood, but it is known to be associated with severe stress, one cause of which may be extreme physical exertion. The possibility that hunting of red deer by hounds might lead to myopathy in those that escaped was suggested by Henshaw & Allen (1989). The effects of capture have been studied in other deer, and a large-scale study of white-tailed deer (*Odocoileus virginianus*) in the USA suggested that 12% of the deer might die within 26 days after being captured with a rocket net (Beringer *et al.* 1996).

The cortisol concentrations were much higher in hunted animals than in non-hunted animals; the concentrations in non-hunted deer were low, but not significantly different from undisturbed deer studied by others (e.g. Smith & Dobson 1990; Carragher *et al.* 1997; Ingram *et al.* 1997). Hunting produced bigger effects than most other stressors applied to red deer (e.g. Smith & Dobson 1990; Carragher *et al.* 1997; Diverio *et al.* 1996; Jago *et al.* 1997). Indeed, the concentrations were as high as have ever been observed in red deer, even when challenged with adrenocorticotrophic hormone (ACTH), which triggers a maximal release of cortisol (Bubenik & Bartos 1993; Goddard *et al.* 1994; Ingram *et al.* 1997). The patterns of the hunts in which deer repeatedly dashed away from hounds, only to be found again, meant that these deer were successively triggered into flight. This may explain why cortisol concentrations were so high in hunted deer. Cortisol is commonly referred to as a 'stress hormone' because its secretion is associated with many types of physical and emotional challenge, such as exercise, social isolation, loss of social status and hypoglycaemia (Broom & Johnson 1993); exercise alone typically does not lead to maximal response unless accompanied by other challenges (Mason 1971). Cortisol concentrations elevated to the extremes observed in the hunted deer provide a strong indicator of great physiological and psychological stress.

In humans, at least, what is assayed as β-endorphin, which has opioid activity in the brain, may be β-lipotrophin (Gibson *et al.* 1993), which does not have opioid activity; β-lipotrophin has the same precursor as β-endorphin and ACTH, which stimulates synthesis of cortisol, but release in hunted deer of what was assayed as β-endorphin was not strongly associated with cortisol. Although high concentrations in the plasma of 'β-endorphin' are often associated with physiological and psychological stress in other species (Broom & Johnson 1993), the functional significance outside the brain is poorly understood. It is of interest, therefore, that in the hunted deer 'β-endorphin' was strongly correlated with CK concentration.

Our results provide strong evidence of damage to red blood cells. The presence of haem in the plasma, which coloured it deeply, was not due to post-mortem events because (i) haem concentrations were not related to the time from collection of blood to centrifugation; (ii) the hunted animals differed systematically from the non-hunted animals, whereas the methods of collection, in as much as they differed, would have suggested greater post-mortem haemolysis in the non-hunted animals; (iii) haem was not correlated with potassium, which would have been expected if red blood cells broke up after death; and (iv) haem was correlated with bilirubin. Bilirubin, which is a breakdown product of haemoglobin *in vivo*, was positively correlated with haem in both hunted and non-hunted deer. This finding strongly suggests that most haem release occurred before death.

Some of the haem may have been due to myoglobin rather than haemoglobin, and haem concentrations were correlated with concentrations of CK in the hunted animals. However, CK concentrations were correlated with the extent of the hunt, whereas haem concentrations were not. A simple model that would explain the results is that haemolysis occurred at an early stage in the hunt, and the haemoglobin released into the blood was steadily converted to bilirubin. In longer hunts, myoglobin from ruptured muscle fibres was released into the plasma in increasing quantities. As a consequence, according to this model, the concentration of haem, which reflects both haemoglobin and myoglobin, would not be related to the length of the hunt.

What might have caused the initial haemolysis? Exercise, even of short duration, alters the properties of erythrocytes (Smith *et al.* 1997), and can be involved in haemolysis in horses (Avellini *et al.* 1995). Red deer have small erythrocytes (Hawkey & Dennett 1989) that are known to be osmotically fragile; changes in ionic concentration may lead to swelling and lysis (Whitten 1967; Jain 1986). Sodium concentrations were significantly higher in hunted than in non-hunted deer, and were not correlated with the extent of the hunt. Initially, chloride concentrations were not elevated, but were significantly correlated with the extent of the hunt. All hunted deer may have passed through a period when their blood became highly acidic. It is possible that animals that were chased for longer distances did not show an initial peak in acidity. However, hunted deer that were observed directly began with a burst of galloping which was likely to have led to a rapid rise in lactate concentrations, high acidity and, therefore, a low pH. Another possibility which we were unable to test was that haemolysis resulted from hyperthermia. While some haemolysis has been associated with intense exercise and percussion, the concentrations of plasma haemoglobin and total bilirubin in hunted red deer indicate intravascular haemolysis of a severity not found in other species (Weight *et al.* 1991; Robertshaw & Swaminathan 1993). Bilirubin concentrations fluctuated with season, being particularly high in the autumn, indicating that naturally occurring changes in state in red deer may also make them more susceptible to haemolysis than is normal in other species.

Creatinine and sodium concentrations indicated that the blood of the hunted animals was significantly more concentrated than the blood of the non-hunted animals. These changes had already occurred in the least extensive hunts and did not vary with the extent of the hunt. Such concentration of the blood as a result of heightened activity is well known in many species, and functions to increase efficiency of glucose transfer to muscles (Hoppeler & Billeter 1991). That no further concentration occurred in more extensive hunts suggests that the deer did not suffer from dehydration as a result of prolonged chases. Their behaviour indicates that, even though they have sweat glands (Jenkinson 1972*b*), panting is a more important form of evaporative cooling than sweating (Jenkinson 1972*a*). The disadvantage for the deer is that they may seriously overheat as a result of prolonged exertion (McAllum 1985).

In conclusion, the exertion associated with hunting with hounds resulted in marked physiological distur-

bances of red deer, including muscle damage and pronounced intravascular haemolysis. We do not believe that these changes merely occurred at the ends of the hunts. The evidence suggests that haemolysis occurred early in the hunt, resulting perhaps from upsets in ionic balance, extreme plasma acidity or hyperthermia. Other evidence points to the cumulative effects of hunting: for instance, leakage of muscle enzymes into the bloodstream was greater in more extensive hunts. We were not able to dissociate various factors that may have separately or jointly contributed to the observed concentrations of substances in the plasma. Nevertheless, this study provides the first quantitative evidence that the physiological effects of hunts of even a relatively short distance and duration are severe, while longer hunts are characterized by signs of extreme exhaustion. Physiological changes of this nature are uncommon in both human and non-human athletes, and would not be expected to result from the typically short chases of red deer by wolves in natural conditions.

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