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Episodic and seasonal rhythms of cortisol secretion in male Eld's deer (*Cervus eldi thamin*)

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ABSTRACT

Episodic and seasonal rhythms of cortisol secretion were evaluated in six adult Eld's deer (*Cervus eldi thamin*) stags. Plasma cortisol was measured in serial blood samples collected via remote catheterization every 10 min for 10 h within 2 weeks of the summer solstice (21 June), autumn equinox (22 September), winter solstice (21 December) and spring equinox (20 March), and in weekly blood samples collected from sedated stags. Cortisol was secreted episodically at a rate of approximately 0·6 peaks/h. Based on quantitative peak detection analyses of each 10-h data series, no overall seasonal differences ($P > 0·05$) were detected in the number of peaks (mean range, 5·7–6·2), maximal peak height (mean range, 30·1–40·8 nmol/l), mean peak height as per cent increase (mean range, 158–168%), mean interval between peaks (mean range, 80·1–88·6 min), mean peak width (mean range, 55·1–65·1 min) and mean peak area under the curve (mean range, 675–816 nmol/l min). Based on weekly blood sampling, spring cortisol concentrations were elevated ($P < 0·05$) compared with summer and autumn concentrations. However, when mean cortisol concentrations derived from the 10-h quarterly data sets were analysed, no seasonal differences ($P > 0·05$) were detected. The present study represents the first detailed confirmation of episodic cortisol secretion in any cervid. Results (1) indicate that Eld's deer stags lack a distinct seasonal rhythm of cortisol secretion and (2) clearly illustrate the need for frequent blood sampling in fully conscious individuals to ensure accurate assessment of adrenal status in cervids.

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INTRODUCTION

Daily rhythms of cortisol secretion have been described in man (Weitzman *et al.* 1971), rhesus monkeys (Holaday *et al.* 1977), cattle (cows, Fulkerson *et al.* 1980; bulls, Thun *et al.* 1981), sheep (ewes, McNatty *et al.* 1972; rams, Kennaway *et al.* 1981), sows (Bate & Hacker, 1985; Griffith & Minton, 1991) and mares (Johnson & Malinowski, 1986). In these species, cortisol is secreted episodically, superimposed upon a diurnal rhythm of high daytime and low evening/night-time concentrations. However, other species such as the dog (Thun *et al.* 1990), white-tailed deer (Bubenik *et al.* 1983) and rusa deer (van Mourik & Stelmasiak, 1984*a*) apparently lack a diurnal rhythm of cortisol secretion. Reports describing seasonal cortisol secretory rhythms are limited; however, no distinct seasonal differences in cortisol secretion have been observed in rams (Kennaway *et al.* 1981), dogs (Thun *et al.* 1990) or white-tailed deer (Bubenik *et al.* 1983).

In white-tailed (Seal & Erickson, 1969; Bubenik *et al.* 1975; Smith & Bubenik, 1990) and rusa (van Mourik *et al.* 1986) deer, cortisol is the predominant adrenal steroid, whereas corticosterone comprises between 15 and 30% of total corticosteroid production. Most studies of adrenal function in deer have been carried out in sedated or fully anaesthetized individuals. Moreover, although several investigations have demonstrated the existence of episodic secretion of pituitary and gonadal hormones in male deer (red deer, Lincoln & Kay, 1979; fallow deer, Asher *et al.*

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1989), including the Eld's deer (Monfort *et al.* 1993), no investigation has detailed the hour-to-hour secretory dynamics of cortisol in any deer species.

Eld's deer (*Cervus eldi thamin*), a sub-tropical species that is sparsely distributed from eastern India to Indochina and southern China (3° to 25° N latitude), exhibit strong seasonal rhythms at northern temperate latitudes (38° N latitude). This subtropical endangered species is unique because it is also seasonal in the subtropics, and fails to exhibit a latitudinal shift in the timing or duration of its annual reproductive rhythms after translocation to northern temperate zones (Monfort *et al.* 1990). We became interested in comparing the interactions between gonadotrophin and steroid hormone secretion in Eld's deer with those documented for cervid species living in temperate zones. It is well-known that increased activation of the hypothalamic-pituitary-adrenal axis can influence the reproductive axis. Like most cervids, Eld's deer are timorous and easily excitable. Thus, we were concerned that stress associated with chronic cannulation and serial blood sampling might adversely affect our ability to study true basal secretory activity. To minimize stress for blood collection, stags were sampled while fully conscious from indwelling cannulae inserted 1 day earlier as well as sedated once weekly for blood collections over a 1-year interval. Our objectives were to: (1) determine whether cortisol secretory patterns could provide an index of animal stress during serial blood sampling; (2) investigate the possible existence of ultradian and/or episodic rhythms of cortisol secretion; and (3) characterize the seasonal pattern of cortisol secretion.

MATERIALS AND METHODS

Animals

Six adult Eld's deer males (2–8 years of age; 70–120 kg body weight) were maintained at the Smithsonian Institution's Conservation and Research Center, Front Royal, Virginia (38° N latitude). Animals were housed individually in indoor stalls (3·4 × 4·6 m with skylights) connected to outdoor enclosures (3·6 × 36·6 m) and exposed to natural fluctuations in photoperiod. Individuals were maintained within visual proximity of other males and within auditory and olfactory proximity of conspecific adult males and females.

Blood sampling

Serial blood samples were collected via remote catheterization every 10 min for 10 h (09.00–19.00 h) within 2 weeks of the summer solstice (21 June), autumn equinox (22 September), winter solstice (21 December) and spring equinox (20 March). On the afternoon before the day of serial blood sampling, each male was sedated using xylazine (Rompun, Mobay Corp., Shawnee, KA., U.S.A.; 0·5 mg/kg, i.m.), and dual i.v. jugular catheters (16 gauge × 22·9 cm; Centrasil; Baxter Healthcare Corp., Deerfield, IL, U.S.A.) were inserted, sutured in place and connected to a 76 cm extension line (Abbot Hospitals, Inc., Chicago, IL. U.S.A.). Catheters were flushed with heparinized saline (10 ml, 30 IU/ml) to prevent intracannula clotting, and bandaged securely. On the day of blood collection (09.00 h), two animal handlers entered the collection stall, accessed the short extension line (76 cm line) from underneath the bandage, and connected another extension line (150 cm) to permit remote removal of blood from outside the stall. All blood samples were collected while males were fully conscious (at least 18 h after the previous day's anaesthesia) in a 1·8 × 0·9m stall bedded with straw where they were free to stand, recline and turn around comfortably. To obtain each blood sample, a syringe attached to a three-way stopcock was used to aspirate 0·9% (w/v) NaCl through the line until undiluted blood was collected. Each blood sample (~3 ml) was placed into a heparinized tube on ice, centrifuged (1500 ***g***) within 2 h, and the plasma was stored at −70 °C until assayed. The extension line was then flushed with either 25 ml heparinized (5 IU/ml) or non-heparinized saline on alternate blood draws to prevent clot formation. Alfalfa hay and water were available *ad libitum* during blood sampling. Weekly blood samples were collected by jugular venepuncture (10 ml) from each male after xylazine hydrochloride (0·3–0·5 mg/kg, i.m.) sedation. After a clotting time of 2 h at 4 °C, serum was recovered and stored frozen (−70 °C) until assayed.

Cortisol radioimmunoassay

Serum and/or plasma cortisol was measured using a double-antibody solid-phase ^{125}Iradioimmunoassay kit (Coat-A-Count, DPC, Los Angeles, CA, U.S.A.). Serial dilutions of plasma or serum yielded displacement curves parallel to that obtained with the standard preparation. The mean recovery of cortisol (range, 14–1380 nmol/l) added to pooled Eld's deer plasma was 99·6 ± 5·4% ($y = 1{\cdot}15x - 1{\cdot}67$; $r^2 = 0{\cdot}99$; $P < 0{\cdot}001$). Assay sensitivity was 14 nmol/l, and the intra-assay variation was <10%. Interassay coefficients of variation for two separate internal controls were 4·4% and 6·9%. High-pressure liquid chromatography separation (Monfort *et al.* 1991) of extracted (10:1, dichloromethane:plasma) Eld's deer plasma and radioimmunoassay of collected fractions revealed that >95% of cortisol immunoreactivity was recovered in comparison with pre-chromatographed plasma. All immunoreactivity was associated with a single peak that co-eluted with [^{3}H] cortisol added before chromatography as a co-chromatographic marker.

Statistical analyses

Hormone concentrations are represented as means ± S.E.M. All statistical comparisons were performed using Statview 512+ (BrainPower, Inc., Calabasas, CA, U.S.A.) on a Macintosh computer. Between-season comparisons of weekly mean cortisol concentrations and peak detection parameters were determined by repeated measures analysis of variance. Multiple pairwise contrasts were performed using Fisher's protected least-significant difference test. Within-season comparisons between mean cortisol concentrations derived from weekly and serial blood collections were performed using a two-tailed Student's *t*-test. For this analysis, weekly hormone concentrations for each individual were partitioned by seasons into summer (June–August), autumn (September–November), winter (December–February) and spring (march–May) according to the date of sample collection. Mean seasonal concentrations were then compared with the corresponding mean concentrations derived from summer, autumn, winter and spring serial blood collections.

For 10-h serial sampling periods, cluster analysis (Veldhuis & Johnson, 1986; version 5·0), a statistically based peak detection algorithm, was used to assess pulsatile cortisol secretion. A 2 × 2 (two nadir points × two peak points) cluster size was used for cortisol in combination with a constant 10% coefficient of variation and pooled *t* statistic of 2·0. The cluster configuration and *t* statistic were selected to limit the false peak detection rate to ~5% (Veldhuis & Johnson, 1986). This program estimated all possible increases in the data series when successive nadir clusters (two data points) were compared against immediately successive potential peak regions (two data points). All significant decreases within the data series were also analysed with reference to this particular cluster configuration. The maximum peak height was calculated as the largest peak amplitude expressed as the absolute value (nmol/l) of the concentration achieved within the largest peak. The mean peak height as a percentage increase represents the percentage increase in the mean peak amplitude above preceding nadir values. The mean peak width was defined as the mean time elapsed in minutes between the first significant upstroke and first significant downstroke within the peak. The mean interval between peaks was also expressed in minutes. Mean peak area was the average of the areas under each detected peak; area was calculated as the product of the peak amplitude (nmol/l) multiplied by the peak duration (min).

RESULTS

Episodic cortisol secretory profiles from six males collected for 10 h (six samples/h) during the spring (*a–c*), winter (*d–f*), autumn (*g–i*) and summer (*j–l*) are presented in Figs. 1 and 2. Quantitative peak detection analyses for each 10-h data series are summarized in Table 1. No within- or among-subject seasonal differences were detected in any of the pulse parameters examined, and no apparent diurnal secretory rhythms were observed. Overall mean values for peak detection parameters for all seasons combined were: number of peaks = 5·95 ± 0·42/10 h (0·6 peaks/h); maximal peak height = 37·0 ± 5·8 nmol/l; mean peak height as per cent increase = 164·3 ± 6·0%; mean interval between peaks = 84·9 ± 2·1 min; mean peak width = 58·1 ± 2·9 min; and mean peak area = 742·1 ± 79·7 nmol/l min.

In unanaesthetized Eld's deer, cortisol concentrations were occasionally elevated at the beginning of bleeding sequences, presumably as a result of transient stress associated with accessing the catheter extension line. This is illustrated in Fig. 2*d* where cortisol levels began near 135 nmol/l, but as the bleeding series progressed, pulsatility was maintained as hormone concentrations declined towards baseline. In another deer (Fig. 1*j*), the catheter became kinked, so it was necessary for an animal handler to enter the stall (see asterisk) and reposition the catheter during removal of blood. As a result, the animal became notably agitated, and cortisol concentrations increased from 15 to 165 nmol/l over 60 min. The deer depicted in Fig. 2*c* became suddenly aggressive 8 h into the sampling series (see asterisk), inexplicably butting his antlers against the side of the stall. This aggressive behaviour was associated with an increase in cortisol from 15 to 135 nmol/l within 10 min. A single representative cortisol profile is presented in Fig. 3 with the animal's activity pattern superimposed. A steady episodic secretory pattern was observed while the animal was fully conscious, standing, positioned in sternal recumbency, eating or sleeping.

Mean (± S.E.M.) weekly cortisol concentrations for six Eld's deer males are shown in Fig. 4. Cortisol concentrations were highest ($P < 0·05$) in the spring compared with the summer and autumn; winter and spring cortisol concentrations were similar ($P > 0·05$). In contrast, no seasonal differences ($P > 0·05$) were observed in cortisol concentrations when means were

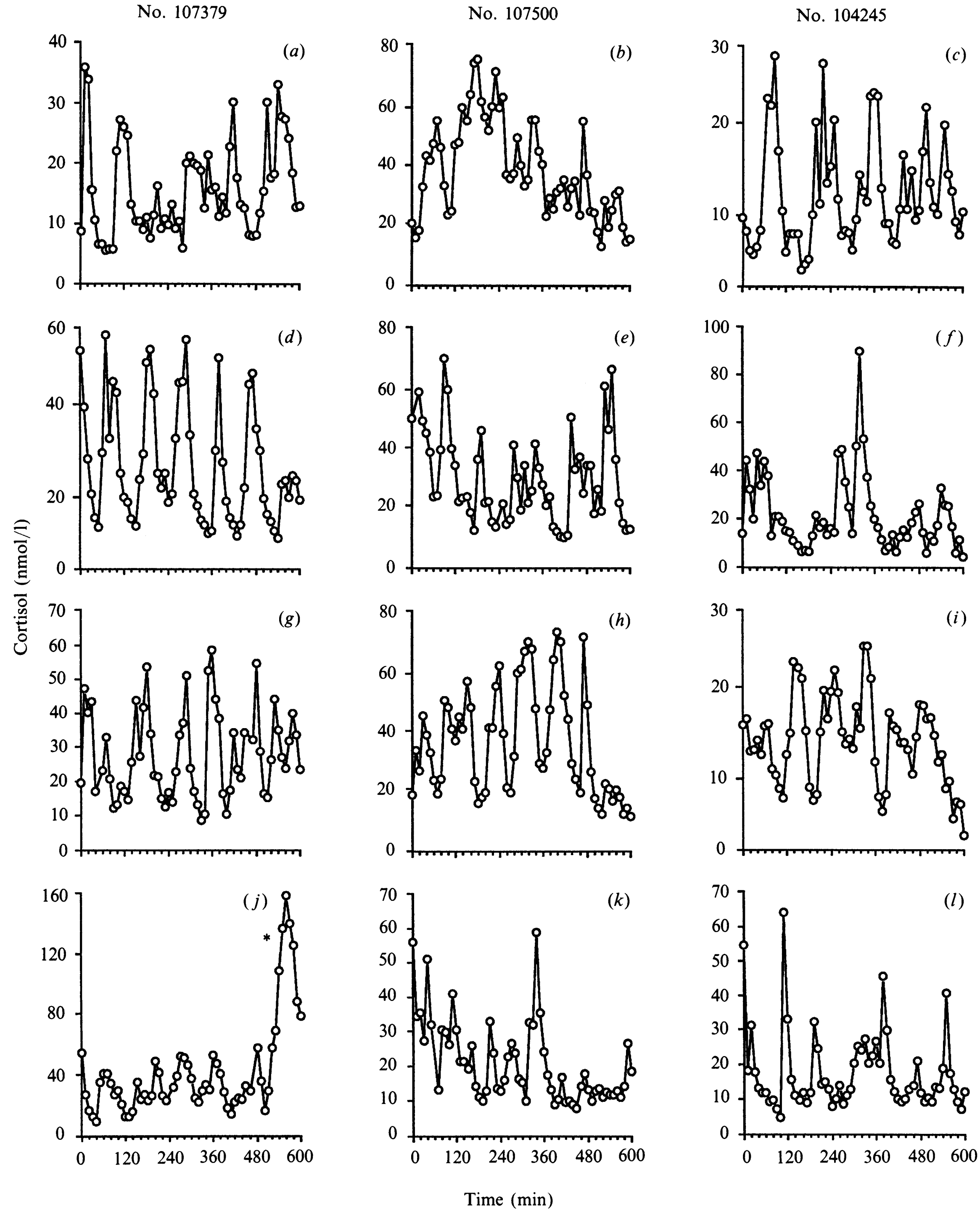

FIGURE 1. Individual cortisol secretory profiles from three stags collected for 10 h (six samples/h) during (*a–c*) the spring, (*d–f*) winter, (*g–i*) autumn and (*j–l*) summer. An asterisk (*j*) denotes an increase in cortisol that occurred while a technician entered the animal's stall to re-position a kinked jugular catheter as blood was sampled. Note different scales for cortisol concentrations among animals and seasons.

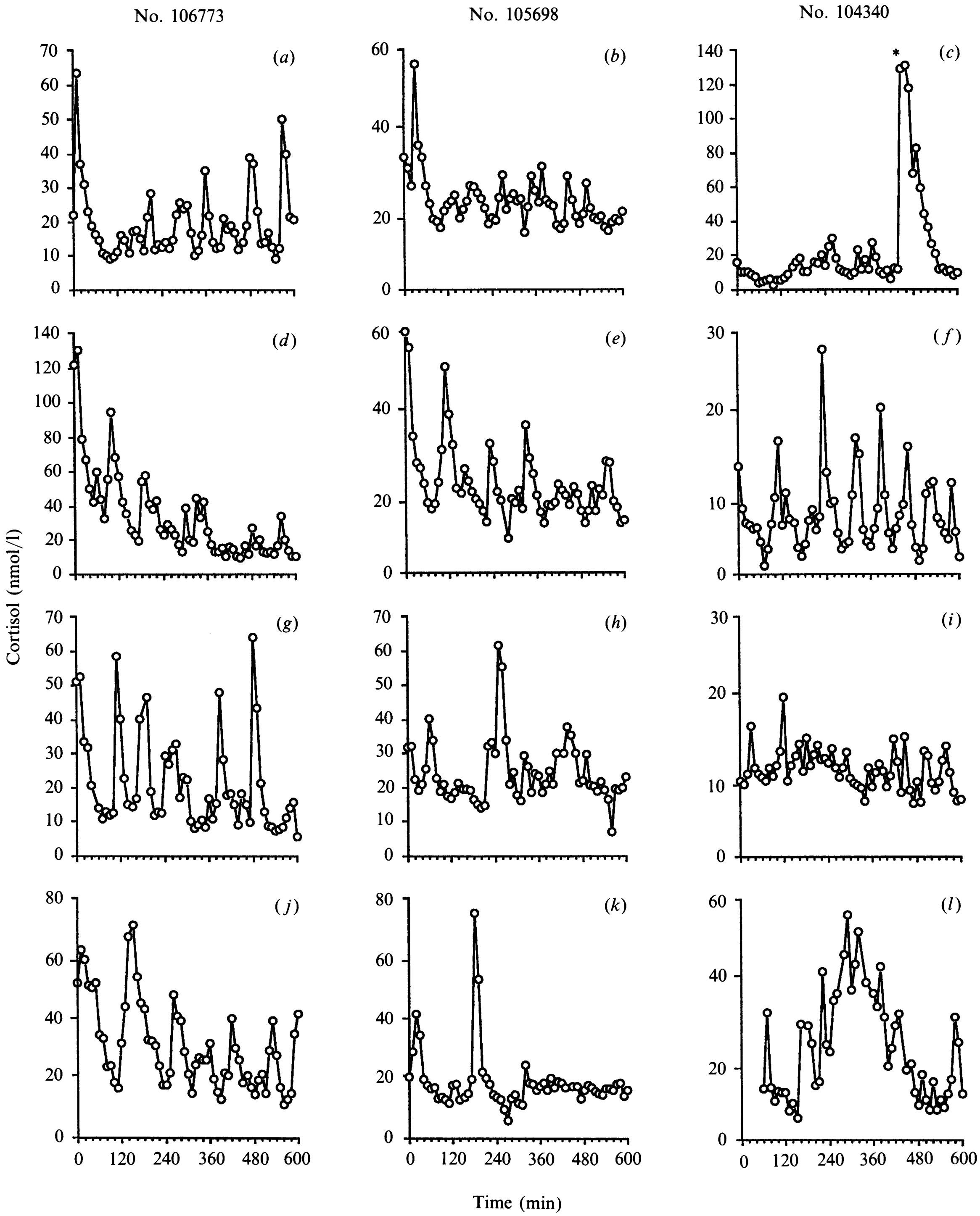

FIGURE 2. Individual cortisol secretory profiles from three stags collected for 10 h (six samples/h) during (*a–c*) the spring, (*d–f*) winter, (*g–i*) autumn and (*j–l*) summer. An asterisk (*c*) represents an increase in cortisol that was associated with aggressive behaviour exhibited by this stag during the blood-sampling interval.

TABLE 1. Mean (±S.E.M.) quantitative peak detection parameters derived from cluster analysis, a statistically based peak detection algorithm, for six stags sampled for 10 h at 10-min intervals during each season

	Summer	**Autumn**	**Winter**	**Spring**
No. peaks/8 h	5·8 ± 10·6	6·2 ± 0·5	5·7 ± 0·2	6·2 ± 0·4
Maximum peak height (μg/l)*	14·8 ± 1·1	14·0 ± 2·9	14·0 ± 2·0	10·9 ± 2·2
Mean peak height as % increase ($n = 6$)	158·4 ± 9·0	167·7 ± 13·7	168·4 ± 12·0	162·6 ± 15·0
Mean interval between peaks (min)	87·5 ± 4·0	88·6 ± 2·7	83·6 ± 4·3	80·1 ± 6·0
Mean peak width (min)	65·1 ± 7·1	56·3 ± 5·4	56·1 ± 6·8	55·1 ± 3·7
Mean peak area (nmol/l min)	295·8 ± 43·5	272·2 ± 75·3	264·2 ± 56·4	244·8 ± 65·8

All within-row means are similar ($P > 0·05$).
*Peak height represents the absolute value of the maximal concentration attained within the largest peak.

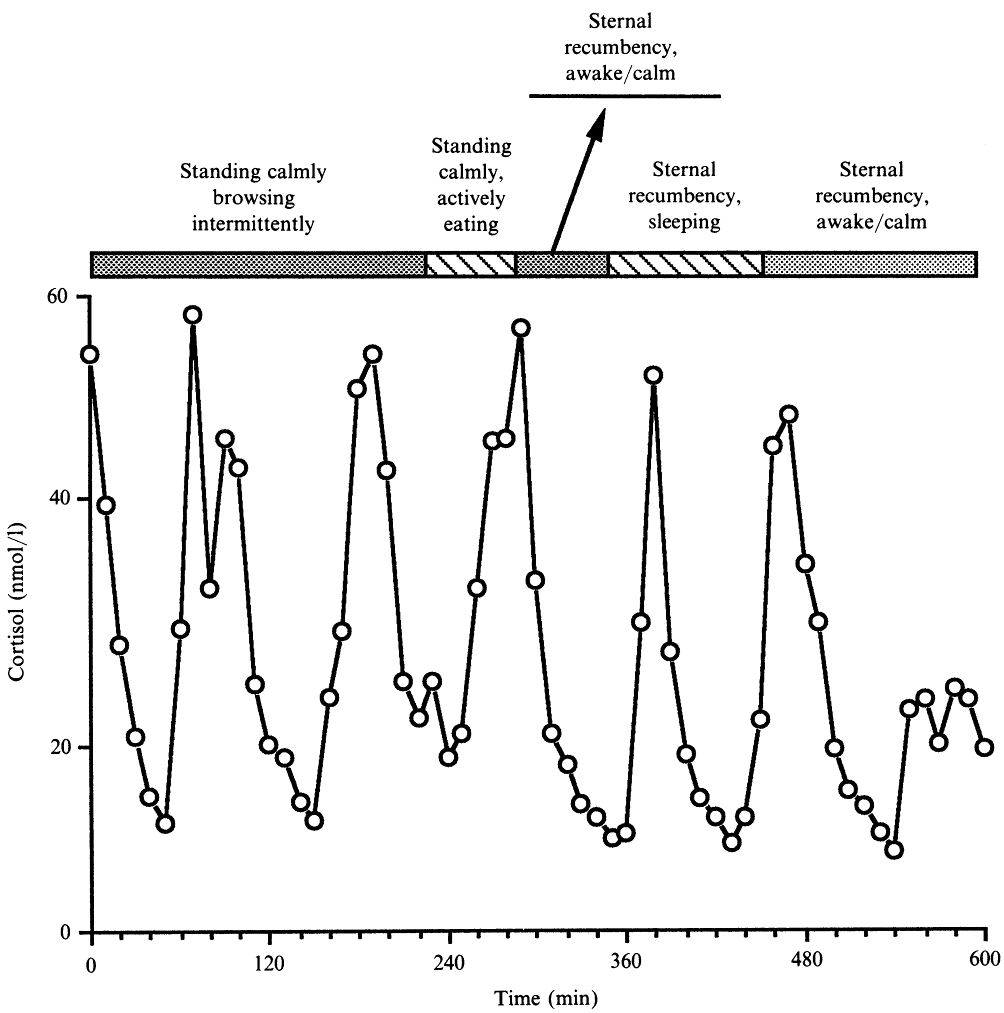

FIGURE 3. Individual cortisol secretory profile from a single representative Eld's deer stag (no. 107379 in winter) with superimposed activity patterns.

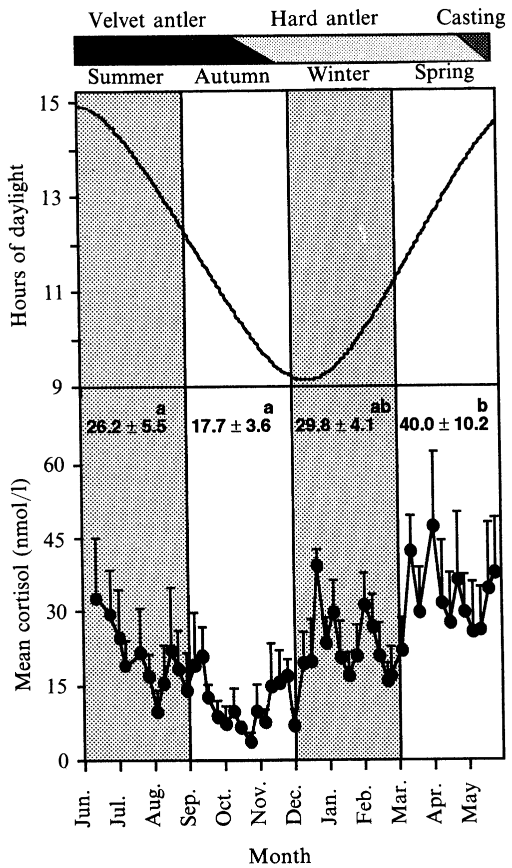

FIGURE 4. Mean (±S.E.M.) weekly and seasonal cortisol concentrations in six sedated Eld's deer stags. The hours of daylight and a schematic representation of the relative timing and stages of the antler cycle (adapted from Monfort *et al.* 1993) are depicted in the upper panel. Means with different superscripts differ ($P<0{\cdot}05$, repeated measures ANOVA combined with multiple pairwise comparisons using Fisher's protected least-significant difference test).

derived from the quarterly 10-h serial blood collections (Fig. 5). There were no significant ($P>0{\cdot}05$) within-season differences between mean hormone concentrations derived from weekly and serial quarterly blood collections.

DISCUSSION

Although the episodic nature of cortisol secretion has been described in several ruminant species, this study represents the first evidence for episodic cortisol secretion in any cervid species. Our results indicate that Eld's deer generally exhibited 0·6 cortisol peaks/h with peaks that increased above basal levels by ~150%. Cortisol pulse frequency in Eld's deer was similar to that of man (0·4 peaks/h; Weitzman *et al.* 1971; Vance & Thorner, 1989), cows (0·2–0·6 peaks/h, Fulkerson *et al.* 1980; Thun *et al.* 1981), sheep (0·2–0·5 peaks/h; Fulkerson & Tang, 1979; Kennaway *et al.* 1981) and dogs (<0·1–0·3 peaks/h; Thun *et al.* 1990).

During times of apparently normal undisturbed activity, the rhythm of cortisol secretion in Eld's deer consisted of repeated 60-min surges that returned to baseline concentrations between peaks. However, our data suggested that acute alterations to this established episodic rhythm could be related to periods of presumed behavioural stress and/or aggression. Interestingly, these perturbations appeared to affect pulse amplitude to a greater extent than pulse frequency. We do not know if such increased cortisol concentrations represented maximal adrenal responsiveness. However, other deer species respond to adrenocorticotrophic hormone (ACTH) challenges with presumably maximal cortisol concentrations of 250–580 nmol/l (white-tailed deer, 275 nmol/l, Seal *et al.* 1983; 410–580 nmol/l, Smith & Bubenik, 1990; rusa deer, 250 nmol/l, van Mourik & Stelmasiak, 1984*b*) within 30–60 min of ACTH administration. Episodic cortisol secretion presumably reflects the secretion of hypothalamic releasing hormones and pituitary ACTH. Such a rhythm may prevent down-regulation of the adrenal axis and maintain it in a highly primed condition for responding to external stressors. Differences in basal cortisol concentrations may vary among individuals, but pulse frequency is relatively consistent, and stags respond rapidly to external stressors by superimposing high-amplitude cortisol surges upon basal hormone pulses. If the role of episodic cortisol secretion is to enhance adrenal activation, then such rhythms may be more distinct in highly excitable species such as cervids where effective fight-or-flight reactions are critical for animal survival.

Basal cortisol concentrations in unanaesthetized Eld's deer (15–40 nmol/l) were similar to those reported for unanaesthetized or anaesthetized rusa deer (<15 nmol/l; van Mourik & Stelmasiak, 1984*a*,*b* respectively), but generally were lower than in white-tailed deer that were manually restrained (135–210 nmol/l; Seal *et al.* 1983) or sedated (25–275 nmol/l; Bubenik *et al.* 1983; Smith & Bubenik, 1990). No apparent trend towards a diurnal rhythm was observed during the sampling interval (09.00–19.00 h); however, sampling duration was insufficient to exclude the existence of a diurnal secretory pattern in this species confidently. Interestingly, no diurnal rhythm of cortisol secretion has been

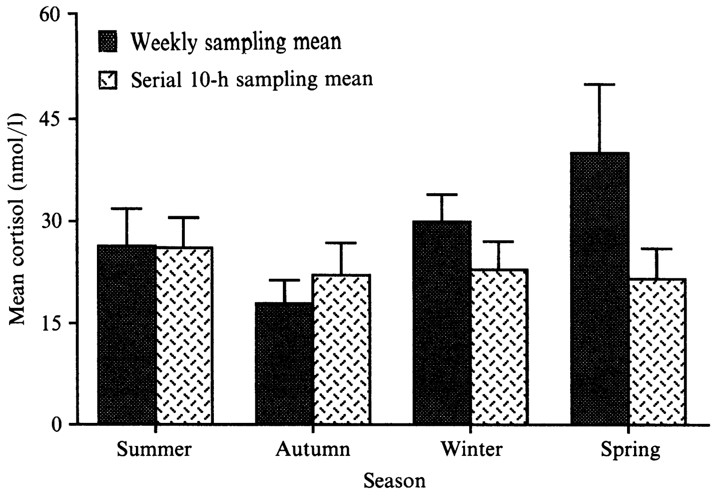

FIGURE 5. Mean (±S.E.M.) seasonal cortisol concentrations derived from weekly and serial blood samples from six stags. Within-season differences were not significant ($P > 0{\cdot}05$, Student's t-test).

detected in either white-tailed (Bubenik *et al.* 1983) or rusa (van Mourik & Stelmasiak, 1984*a*) deer.

There are conflicting reports regarding the existence of seasonal rhythms of cortisol secretion in white-tailed deer which have been reported to remain unchanged (Bubenik *et al.* 1975) or higher during the autumn rutting season (Bubenik *et al.* 1983; Bubenik & Leatherland, 1984). In white-tailed deer, seasonal cortisol secretion is more evident in calm bucks (Bubenik & Leatherland, 1984), whereas cortisol concentrations are elevated throughout the year in excitable males (Bubenik *et al.* 1983). Interestingly, based upon weekly blood collection data from sedated animals, it appeared initially that Eld's deer exhibited a seasonal rhythm of cortisol secretion, with higher levels during the pre-rut and rutting seasons. However, when the mean cortisol concentrations from the serial 10-h sampling intervals were compared, seasonal differences disappeared. Not only were mean hormone concentrations similar among seasons, but no seasonal change in the dynamics of cortisol secretion was observed. Perhaps the earlier suggestion of seasonal variations in cortisol production in white-tailed deer were biased because of the episodic secretory nature of cortisol secretion and insufficient sampling frequency. Together, these data illustrate the importance of analysing multiple blood samples for assessing adrenal status in deer accurately.

Most cervids are highly excitable, and handling, anaesthesia and/or individual differences in temperament may all acutely influence cortisol secretion. For example, xylazine sedation is commonly used to obtain blood samples from deer, and this anaesthetic acutely increases cortisol concentrations in both white-tailed (Chao *et al.* 1984) and rusa (van Mourik & Stelmasiak, 1984*a*) deer. In the present study, Eld's deer were also sedated with xylazine for weekly blood collection. Because higher dosages of xylazine were required to obtain adequate sedation in Eld's deer during the pre-rut and rutting seasons (S. L. Monfort, personal observations), this may have accounted for the 'apparent' seasonal pattern of cortisol secretion in sedated deer. Bubenik *et al.* (1983) have also reported the use of higher xylazine dosages in 'excitable' males that exhibited erratic seasonal cortisol rhythms. It is possible that seasonal rhythms in cortisol secretion observed in restrained and/or anaesthetized subjects may reflect differential sensitivity of the hypothalamic-pituitary-adrenal axis to anaesthetics and/or the 'stress' associated with handling during different seasons.

Seasonal patterns in activity, behaviour and metabolism are well documented in cervids (Loudon & Brinklow, 1992). Eld's deer also exhibit well-defined seasonal fluctuations in body weight, morphometry, testicular size, antler growth and voluntary food intake (Monfort *et al.* 1993). Therefore, our data suggest that cortisol alone does not provide a useful index of the changes in the seasonal metabolic cycle of this species.

Our results clearly illustrate the need for frequent blood sampling to ensure accurate assessment of adrenal status in cervids. As an indicator of 'stress', cortisol may only be appropriate if the secretory dynamics, including pulse amplitude and frequency are evaluated. Such evaluations would require repeated blood sampling at 10- to 20-min intervals for at least several hours in fully conscious individuals. In Eld's deer, deviation from a regular episodic secretory pattern may suggest physiological stress, whereas infrequent blood sampling, especially when animals are physically restrained or anaesthetized, could easily lead to erroneous conclusions regarding adrenal status.

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