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Brief Communication

The Effect of Blood Sampling on Plasma Cortisol in Female Reindeer *(Rangifer tarandus tarandus* L*)*

A major response of the body to stress is the secretion of adrenocorticotrophic hormone (ACTH) by the adenohypophysis resulting in increased blood cortisol levels. Corticotropin releasing factor (CRF) controls the release of ACTH, mediating a direct effect at the level of the pituitary (*Guillame et al.* 1992). An animal's perception of a stressful event and the resulting increase in cortisol production can be influenced by previous experience, season, time of day, and the animal's sex, age, and condition (*Dantzer & Mormède* 1983, *Moberg* 1985).

Stress and the resulting activity of the hypothalamo-pituitary-adrenal axis (HPA-axis) can have profound effects on reproduction and the immune response (reviewed by *Moberg* 1991 and *Munck et al.* 1984). Cortisol, in addition to CRF and ACTH, can disrupt the endocrine regulation of reproduction imparing those events that occur around estrus and ovulation (*Matteri & Moberg* 1982, *Matteri et al.* 1986, *Moberg* 1985). Administration of ACTH and cortisol are reported to inhibit estrus in gilts and cattle (*Barb et al.* 1982, *Stoebel & Moberg* 1982) and to reduce gonadotropin responsivness to GnRH in rams and cattle (*Matteri et al.* 1984). If long-term stress causes suppression of luteinizing hormone (LH), the animal's reproductive success could be impaired (*Matteri & Moberg* 1982, *Fuquay & Moberg* 1983).

Glucocorticoids are generally considered to be anti-inflammatory and to suppress the immune system (reviewed by *Munck et al.* 1984). Elevation of cortisol over a long period of time suppresses the immune response through decreased antibody production, lymphocyte blastogenesis, circulating lymphocyte count, and leucocyte phagocytosis in lamb and cattle (*Roth & Kaeberle* 1983, *Collins & Suárez-Guémes* 1985).

The use of semi-domestic reindeer in observational studies that involve frequent blood sampling most probably subjects the animals to stressful situations. The aim of this study was to investigate the response in the HPA-axis to physical restraint and blood sampling by jugular venipuncture in adult female reindeer and calves.

Two mature, cycling female reindeer and 3 prepubertal calves were taken from the mountains of Southern Norway in late August. The animals were allowed to move freely together with other animals in an outdoor paddock at the Agricultural University of Norway, Ås . The animals were given free access to food which consisted of grass, lichens and, concentrates. During the autumn, the ration was gradually changed from grass as the

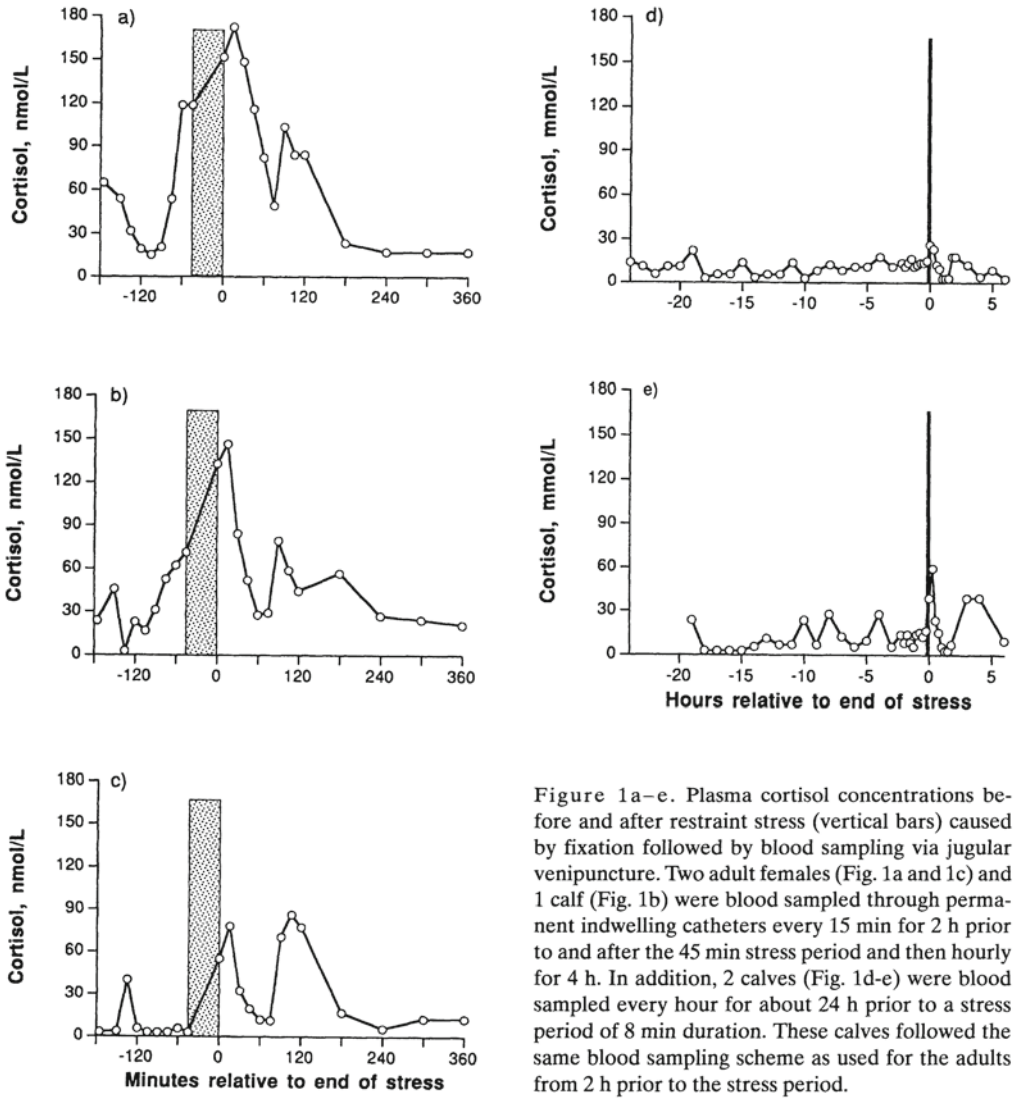

Figure 1a–e. Plasma cortisol concentrations before and after restraint stress (vertical bars) caused by fixation followed by blood sampling via jugular venipuncture. Two adult females (Fig. 1a and 1c) and 1 calf (Fig. 1b) were blood sampled through permanent indwelling catheters every 15 min for 2 h prior to and after the 45 min stress period and then hourly for 4 h. In addition, 2 calves (Fig. 1d-e) were blood sampled every hour for about 24 h prior to a stress period of 8 min duration. These calves followed the same blood sampling scheme as used for the adults from 2 h prior to the stress period.

main feed constituent to lichens and concentrates. Body weights were recorded every week.

One month before the experiment started, a permanent indwelling catheter was inserted into a jugular vein (*Rodriguez & Kunavongkrit* 1983). The animals were then transferred from the herd to small pens and kept 2 and 2 together or alone. Blood samples were drawn from the catheter daily to accustom the animals to contact with people and blood sampling. The handling experiment was con-

ducted in late October, starting at 11:00 am. Blood samples were drawn from the indwelling catheters into heparinized vacutainers every 15 min for 2 h before and after handling, and then every h for 4 h (Fig. 1a-c). From 2 calves, blood samples were also collected every hour for 20-24 h prior to handling (Fig. 1d-e).

The handling procedure was designed to imitate the approach commonly used to sample jugular venous blood from captured reindeer. The animals were chased in a 5x20 metres paddock, caught and restrained while blood was drawn from the jugular vein by venipuncture. This sample marked the first sample after handling (hour = 0, Fig. 1).

Three animals (Fig. 1a-c) were let out together with the rest of the herd to take part in the daily blood sampling which lasted for 45 m. The 2 calves, from which blood samples were collected every hour for 20-24 h prior to handling, were chased for approximately 8 min (Fig. 1d-e), and this procedure did not involve contact with other animals. The blood samples were centrifuged immediately and frozen at -70°C until analyzed for cortisol.

Plasma cortisol concentrations were measured by a radioimmunoassay previously validated for turkey plasma by *Simensen et al.* (1978) with the following modifications. The plasma was diluted (1/5) in distilled water and boiled in water for 10 min. The phosphate buffer used for incubation contained 0.2% bovine serum albumin, and the samples were incubated overnight at +4°C.

There was an increase in plasma cortisol levels in all reindeer following handling and blood sampling by venipuncture. This increase was significant when individual averages of the last 3 samples prior to handling were compared with average values of the first 3 samples after handling ($p<0.05$). Peak cortisol concentration, ranging from 26.2 to 172.4 nmol/l, was reached 15 min after the end of handling in all animals except in 1 calf. This calf showed only a slight elevation in plasma cortisol immediately after handling (Fig. 1d). The peak concentration did not exceed maximal values obtained during the 24 h sampling period prior to handling in this animal.

In sheep and cattle, the cortisol response to various acute stressors normally exhibits a pattern of sharp increase, and gradual decline after the stress is removed (*Johnston & Buckland* 1976, *Fulkerson & Jamison* 1982). A similiar, sharp increase in plasma cortisol has also been reported in several deer species (*van Mourik et al.* 1985, *Wood et al.* 1986). In cattle and reindeer, it was found that handling produced higher plasma cortisol concentrations than transport and slaughter (*Hanssen et al.* 1984, *Mitchell et al.* 1988).

In the present study, a second peak in plasma cortisol, ranging from 38.6 to 103.6 nmol/l, was observed 1½-4 h after handling in 4 animals (Fig. 1a-c and 1e). Cortisol reached pre-handling levels (<30 nmol/l) 3-6 h after handling. Pre-handling cortisol levels in this study were equal to that reported in reindeer (*Rehbinder & Edqvist* 1981, *Wicklund et al.* 1994) and generally higher than those found in white-tailed deer (*Bubenik & Leatherland* 1984). The peak cortisol levels found in this study were much higher than those found in restrained penned white-tailed deer (levels around 15-20 nmol/l) (*Seal* 1972).

The wide range of cortisol increments observed upon handling, as well as the magnitude of the 2 cortisol peaks, reflected a highly individual response to the same stressor. Great variation in cortisol values after handling or slaughter of reindeer was also observed by *Rehbinder & Edqvist* (1981).

In 2 of the animals (Fig. 1a-b), plasma cortisol concentrations increased to high levels (118.6 and 62.1 nmol/l, respectivly) before

handling started. The prehandling increases in cortisol could be caused by the act of blood sampling itself, though the sampling was performed in a manner which should minimize stress. The other animals had low plasma cortisol levels when handling started.

There was no apparent circardian pattern in the 24 h profile of the calves in Fig. 1d-e. The cortisol levels oscilliated between 3 and 30 nmol/l. This is in accordance with the findings in white-tailed deer and rusa deer (*Bubenik et al.* 1983, *van Mourik & Stelmasaik* 1984), though a circardian rhythm of plasma cortisol levels has been reported in cattle (*Thun et al.* 1981) and sheep (*Fulkerson & Tang* 1979). The minor increase seen in plasma cortisol in these 2 animals in response to handling may be due to the very short duration of the stress stimulus used for these animals.

In conclusion, the level of plasma cortisol in female reindeer showed a clear response to handling. The high sensitivity and the rapid increase in cortisol after a stress-inducing event such as handling are most likely characteristics of a wild species, which needs to be aware of potential danger and to have a rapid fight-flight-fright response (*van Mourik* 1985). Plasma cortisol levels may serve as a useful indicator of stress in reindeer kept for research purposes. Similiar to other deer species, a circardian rhythm of cortisol was not evident.

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