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Can cortisol be used to assess acute stress in moose?

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Abstract

In this study, the serum concentration of cortisol was measured in 78 hunted moose (*Alces alces*) shot by rifle. All animals died within 5 minutes after being shot and blood samples were collected. Blood levels of cortisol have been used to assess acute stress and evaluate animal welfare in wild animals, but the animals have been influenced by people during physical or chemical restraint. Little is known about physiology of cortisol in free-ranging moose, and studying these animals without disturbing them are not possible. In the present study, serum cortisol concentrations in shot moose were compared to levels in free-ranging moose immobilized with etorphine-acepromazine-xylazine, medetomidine-ketamine or etorphine in order to evaluate cortisol as a parameter for measuring acute stress. The results showed that the mean serum cortisol concentration in shot moose was 43 nmol/L (2SE = 9). There was no significant difference in cortisol concentration in moose immobilized with etorphine-acepromazine-xylazine and shot moose, probably related to the stress lowering effect of xylazine. The reference values of blood cortisol in moose immobilized with medetomidine-ketamine and etorphine were to 3-4 times higher, probably mainly related to drug influence. The present study indicates that serum cortisol cannot be used as the only variable to assess acute stress in moose and that animals instantly killed by shooting might be used to establish baseline ("normal") values of cortisol in this species.

Key words: moose (Alces alces), cortisol, stress, immobilization

Introduction

In biology and medicine, stress refers to the generalized non-specific response of the body to any factor that overwhelms, or threatens to overwhelm, its compensatory abilities to maintain homeostasis (Arnemo & Caulkett, 2007). Stress is a natural and important part of the daily life of animals, and stressors can evoke responses that are beneficial for the survival of the animal, (eustress), or threaten the welfare of the animal by inducing harmful responses and pathological changes, (distress) (Arnemo & Caulkett, 2007). The stress response is a series of extremely complex and interrelated hormonal and neural events, and stress can be induces by numerous agents and physical, chemical, physiological and motional stimuli (Arnemo & Caulkett, 2007). Stressors always increase the secretion of glucocorticoids (cortisol and

corticosterone), and blood cortisol levels have been widely used as *the* indicator of stress in both humans and animals, and the term *stress* has come to mean any event that elicits release of these hormones (Arnemo & Caulkett, 2007). The individual stress response is either directly or indirectly influenced by the hypothalamus that activates the sympathetic nervous system and the hormonal response (Arnemo & Caulkett, 2007). Both neural and hormonal responses are activated to cope with the emergency, and the major neural response to the acute stress is generalized and immediate activation of the sympathetic nervous system, through a massive outpouring of catecholamines (epinephrine and norepinephrine) from the adrenal medulla, known as the fight-flight response (Arnemo & Caulkett, 2007). The sympathetic alarm reaction makes a rapid and intense response to a stressor; the heart rate can be doubled within 3 to 5 seconds, and the arterial blood pressure can be increased to twice normal, within 10 to 15 seconds (Arnemo & Caulkett, 2007). The predominant hormonal response during acute stress is activation of the hypothalamic-pituitary-adrenal axis that stimulates release of glucocorticoid hormones. Different groups of animals secrete different types of glucocorticoids, and ungulates secrete cortisol predominantly (Arnemo & Caulkett, 2007). The major metabolic effect of increased glucocorticoid secretion is to mobilize fuel, and during acute stress, secretion of glucocorticoids may increase as much as 20-fold within 5 – 20 minutes after a stressor has been recognized (Arnemo & Caulkett, 2007). Glucocorticoid secretion by the adrenal cortex is regulated by a negative-feedback system, and this system maintains a relatively constant level of glucocorticoid. This constant secretion is punctuated by alternating bursts of low-level secretion separated by periods of little or no secretion that can be related to the sleep-wake cycle or to seasonal variation possibly related to changes in daylight length, but these variations are small compared with the dramatic increase in glucocorticoid secretion during acute stress (Arnemo & Caulkett, 2007).

Pursuit, capture, restraint, pain, chemical immobilization and anesthesia of free-ranging wild animals, can induce acute stress, and measurement of blood concentrations of glucocorticoids has been used to assess capture stress in free-ranging wild animals (Arnemo & Caulkett, 2007). Reference data for blood cortisol in many wild species are not available and reported hematology and chemistry values are frequently inferred from small sample sizes and animals in captivity, which may not be representative (Rostal et al., 2012). The physiology of cortisol in free-ranging moose is not well understood, and the reported reference values are based on samples from moose chemically immobilized for radiocollaring, health assessment or other purposes (Rostal et al., 2012). In addition to handling, physical restraint and body condition,

anesthetic drugs and method of drug administration can influence the blood level of cortisol (Arnemo & Caulkett, 2007; Arnemo & Ranheim, 1999). The aim of the present study was to compare serum levels of cortisol in hunted moose shot by rifle with established reference values for chemically immobilized free-ranging moose.

Material and methods

Population and study area

Blood samples from 78 moose of different sex and ages were taken in different hunting areas in Sweden during ordinary hunting in September, October and November, between 1998 and 2011. The moose were shot with a high velocity bullet, and blood was sampled from bleeding vessels before or during evisceration by the hunter or an assistant. All animals died within 5 minutes of being shot. In addition to time to death, the time has been recorded from the animal´s death to the blood sample is taken, and the type, degree and duration of disturbance and pursued and hunting with dog before the animal was shot have been registered.
Blood for serum chemistry was collected in 10-ml glass tubes. The tubes with clotted whole blood were sent by post to the Veterinary Clinic at Kolmarden Zoo, where serum was separated and frozen in 2 ml plastic tubes at -25^{o}C for up to 8 years. Analyses were performed at the laboratory at the Department of Physiology at the Swedish University of Agricultural Sciences, Uppsala in 2007 and 2011. Serum cortisol concentration was analyzed by radioimmunoassay using a Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA, USA.
Blood cortisol concentrations from the moose in this study were compared with values from moose immobilized by dart injection with etorphine-acepromazine-xylazine from helicopter, etorphine from helicopter or from a car or on foot, or medetomidine-ketamine from helicopter or from a car or on foot.

Data analyses

The two-sample t-test was used to compare blood cortisol concentrations from shot moose shot and chemically immobilized and to compare cortisol concentrations in undisturbed, shot moose and moose pursued and hunted with dogs for less than and more than 10 minutes, respectively, before being shot.

Results

The blood cortisol concentration of moose shot and dead within 5 minutes compared with published and unpublished reference values from moose immobilized with different methods and drugs are pictured in figure 1.

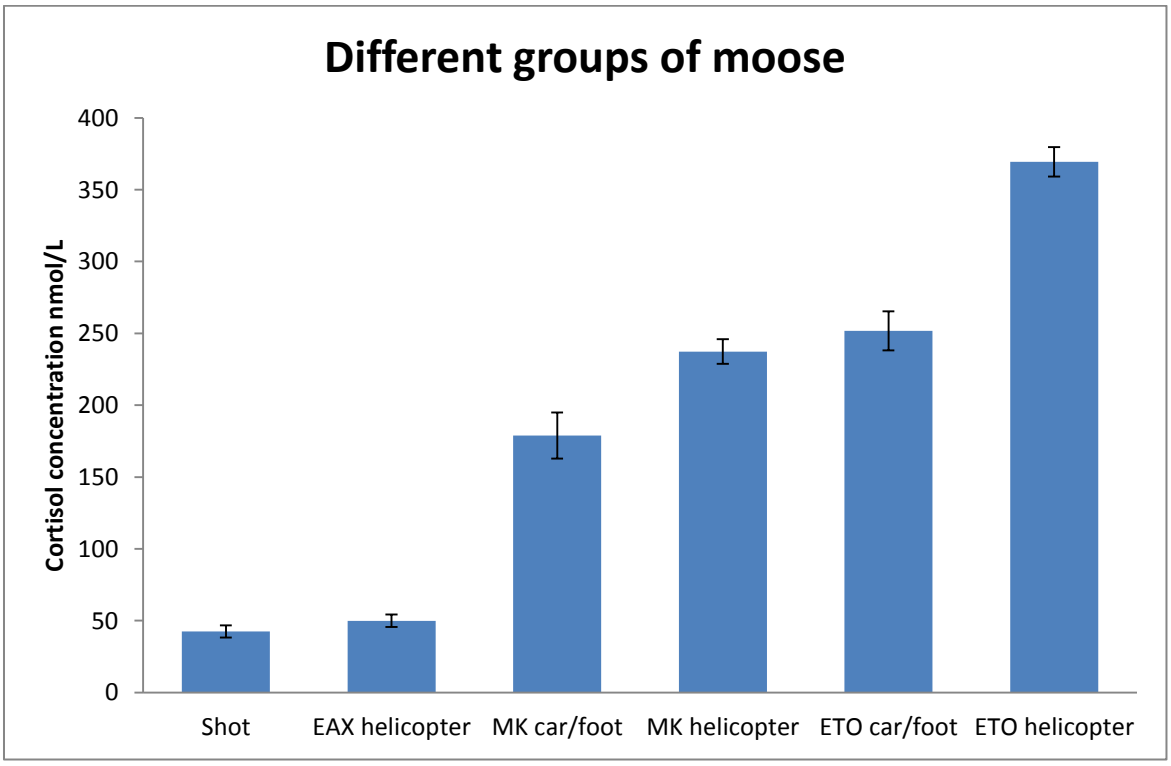

Figure1. Mean cortisol concentration and 2 SE of shot moose dead within 5 minutes (n=78), moose immobilized with etorphine-acepromazine-xylazine (EAX) from helicopter (n=33), moose immobilized with medetomidine-ketamine (MK) from car/on foot (n=27), moose immobilized with medetomidine-ketamine (MK) from helicopter (n=89), moose immobilized with etorphine (ETO) from car/on foot (n=38) and moose immobilized with etorphine (ETO) from helicopter (n=156).

The mean cortisol concentration of 78 moose in this study are 43 nmol/L (2SE = 9). 33 moose immobilized with etorphine-acepromazine-xylazine from helicopter, had a mean cortisol concentration of 50 nmol/L (2 SE = 8) (Lian, 2012), 27 moose immobilized with medetomidine-ketamine from a car or on foot, had a mean cortisol concentration of 237 nmol/L (2 SE =32) (Arnemo, 1995), 89 moose immobilized with medetomidine-ketamine from helicopter, had a mean cortisol concentration of 237 nmol/L (2 SE =17) (Arnemo, unpublished data), 38 moose immobilized with etorphine from a car or on foot, had a mean cortisol concentration of 252 nmol/L (2 SE =27) (Arnemo, unpublished data) and 156 moose immobilized with etorphine from helicopter, had a mean cortisol concentration of 369 nmol/L (2 SE = 21) (Rostal et al., 2012).

The cortisol concentration was significantly higher in moose immobilized from helicopter than with the same drug combination from a car or on foot (etorphine: t=5,38, p<0,0001; medetomidine-ketamine t=3,29, p=0,001).

Based on assessment of disturbance prior to being shot, moose were grouped as undisturbed/unaware of hunter/not pursued; pursued and hunted with dog for less than 10 minutes; and pursued and hunted with dogs for more than 10 minutes. No significant difference was found between the first two groups and the data were pooled.

The cortisol concentrations was significantly higher in moose pursued and hunted with dogs for more than 10 minutes than in undisturbed moose and moose pursued and hunted with dog for less than 10 minutes (t=3,43, p<0,001), see figure 2.

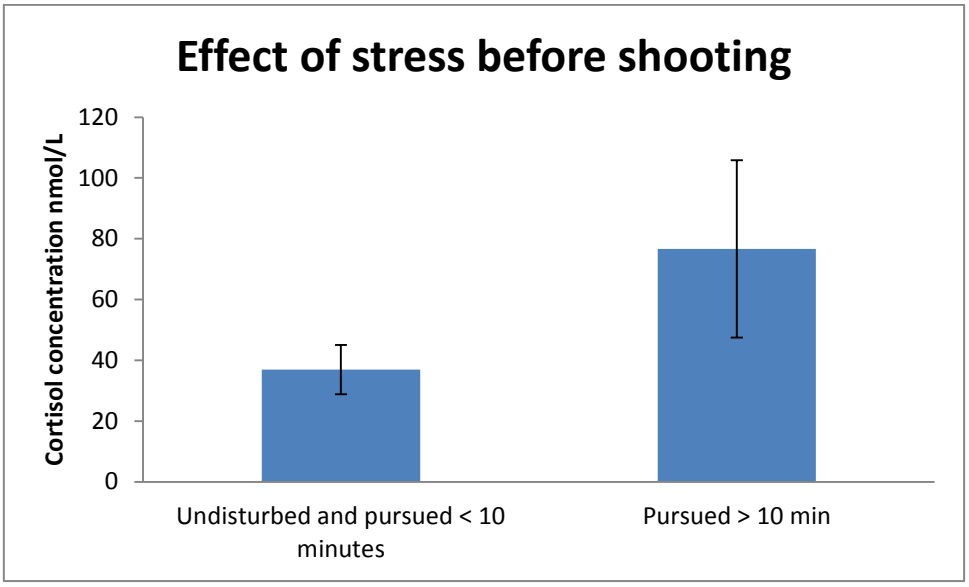

Figure2. Mean cortisol concentration and 2 SE of moose that are undisturbed and pursued for less than 10 minutes (n=67, 2SE=16) and moose that are pursued and hunted with dog for more than 10 minutes before shooting (n=11, 2SE=58).

Discussion

Stress caused by capture and handling of wild animals can be assessed by measuring changes in hematological, serum biochemical and clinical parameters (Mentaberre et al., 2010; Marks, 2010; Pedersen et al., 2009; Morton et al., 1995). Determining normal physiologic data in wild animals are difficult owing to the effect of capture and handling, and the differences in different capture methods (Casas-Díaz et al., 2008). Assessment of stress is important for determining the least stressful method for capturing and handling wildlife species in order to improve animal welfare and to reduce mortality and morbidity (López-Olvera et al., 2009;

DeNicola & Swihart, 1997; Morton et al., 1995). Cortisol has been considered to be the classical stress hormone and have in many studies been used to assess capture stress in free-ranging wild animals (Arnemo & Caukett, 2007; Grigor et al., 1999; Bateson & Bradshaw, 1997; DeNicola & Swihart, 1997; Smith & Dobson, 1990). Animals are known to secrete similar amounts of glucocorticoids during exercise, restraint and mating, and measurement of cortisol alone, cannot be used to differentiate between eustress and distress (Arnemo & Caulkett, 2007). In spite of possible interspecies variation, it's reasonable to assume that closely related *Cervidae* have a similar responses to stress.

Blood cortisol concentration in red deer (*Cervus elaphus*) increase with higher stress level (Bradshaw & Bateson, 2000).The increase in blood glucocorticoid concentration is generally proportional to the intensity of the stressful stimulation, owing to a greater increase in cortisol level in response to severe stress than to mild stress (Arnemo & Caulkett, 2007). Cortisol concentration may increase more when capture is preceded by active pursuit of an animal, for example from a helicopter (DeNicola & Swihart, 1997). Moose immobilized from helicopter seem to be more stressed and have significantly higher blood cortisol concentration than moose immobilized with a less stressful method; approaching from a car or on foot, see figure 1. Cortisol concentration in red deer increase very rapidly about the first 30 min after exposure to stress, before it declines gradually over 30-90 min after the stressor has been removed (Bradshaw & Bateson, 2000; DeNicola & Swihart, 1997; Wesson et al., 1979b). Bateson & Bradshaw (1997) showed that undisturbed red deer, had cortisol concentrations below detectable limits, and that plasma cortisol concentrations increased rapidly after the onset of a stressful stimulus, like hunting, handling and transportation (Bateson & Bradshaw, 1997; Smith & Dobson, 1990). Red deer have significantly higher plasma cortisol concentrations than undisturbed red deer, already 10 minutes after put in restraint (Grigor et al., 1999). The baseline level of cortisol in captive semi-domestic reindeer are probably below 30 nmol/L, but after light restraint and blood sampling by jugular venipuncture plasma levels of cortisol increased to 150 – 180 nmol/L (Sire et al., 1995; Arnemo & Ranheim, 1999). These studies show some of the inherent difficulties of using blood cortisol levels to assess stress. Compared to reference values established for chemically immobilized moose, serum cortisol levels of shot moose, the present study have measured very low levels of blood cortisol concentration in moose in Sweden. The values are up to 3-4 times lower that baseline levels given from chemical immobilized moose in other studies (Rostal et al., 2012; Arnemo, 1995; Arnemo unpublished data).

Some authors point out the importance that blood samples from shot animals should follow rigorous standardization methods as time after death and site of blood sampling to compare samples and results for capturing animals (Wesson et al., 1979a). In the present study on shot moose, recording of time events and collection and handling of samples were carried out by few persons mainly in the same way. According to the results, there are no significant changes of the cortisol concentration and the time for blood sample after death (Holst, 1996). Other studies have shown that the method of collecting and handling the blood sample and the time of blood sampling after death have very little effect on blood chemistry (Bradshaw & Bateson, 2000; Wesson et al., 1979a) and serum cortisol concentration (Holst, 1996), and that cortisol are unaffected by both different stalkers method of killing the deer and the wound site (head/neck or chest) (Bradshaw & Bateson, 2000).

Cortisol levels are influenced by many factors including the baseline level of the species, physiological condition of the individual animal, effects of stressors, pathological conditions, kind of drug and method of drug administration (Arnemo & Caulkett, 2007). Blood parameters are influenced by factors as sex, age and season, and can influence results when comparing heterogeneous groups of animals (Perez et al., 2005; Waid & Warren, 1984; Franzemann 1972). Bradshaw & Bateson, (2000) proved significantly differences in cortisol levels in sex and in deer shot in different habitats. Other studies have proved that factors as area, sex, month or age have small effects on cortisol concentration (Morten et al.,1995; Kie et al., 1983). Anyway; compared to the variation in stress, these variations are small compared with the dramatic increase in glucocorticoid secretion during acute stress (Arnemo & Caulkett, 2007), so in this study, the measures from moose in different sex and ages have been combined.

Different studies have examined the influences of sampling blood with different capture methods, and have proved the profound effects on the blood values measured (DeNicola & Swihart, 1997; Wesson et al., 1979a), and according to Wesson (1979a), it is undesirable to combine and compare blood samples obtained by different methods such as shooting, chemical immobilizing and physical restraint. The results of Wesson (1979a) indicates that shooting may be more satisfactory for sampling stress-related blood measurements such as glucocorticoids than other methods, clean shot is less stressful than physical restraint and the differences between shot and immobilized animals could have been related to both excitement and use of drugs (DeNicola & Swihart, 1997; Perez et al., 2005). Hematological and biochemical data for wild animals use to be sampled under either physical restraint or

chemical immobilization (Rostal et al., 2012; Mentaberre et al., 2012; Casas-Díaz et al., 2008; Arnemo, 1995). On the basis of the plasma cortisol levels in 18 different wildlife spices, Morten et al. (1995) concluded that chemical capture was less stressful than physical restraint, and animals which were tranquilized, had lower than "normal" cortisol levels. Many studies of cervids have shown that several blood parameters, included plasma cortisol are significantly lower after drug immobilization than after physical restraint, explained by the calming effect of the drug and that the restraint method are less stressful (Mentaberre et al., 2010; Morton et al., 1995). The use of tranquilizers may contribute to improve welfare of captured animals in order to reduce stress and prevent its adverse effects (Mentaberre et al., 2010). In addition to handling, physical restraint and body condition, anesthetic drugs and method of drug administration influence the blood level of cortisol, and several studies have shown that different kind of drugs influence the cortisol level in blood (Lian, 2012; Boesch et al., 2011; DeNicola & Swihart, 1997; Arnemo & Ranheim, 1999). The effects of anesthetic drugs of cortisol secretion are very complex, and very little is known about specific effects on glucocorticoid secretion in wild animals, and of other sedative or anesthetic agents than α- 2 adrenoceptor agents (Arnemo & Caulkett, 2007). α- 2 adrenoceptor agonists such as xylazine and detomidine are known to reduce the stress response, evidenced by decreased serum cortisol, but medetomidine, a more potent α- 2 adrenoceptor agent, increases the secretion of cortisol in species such as reindeer and moose (Arnemo & Caulkett, 2007; Arnemo & Ranheim, 1999).

According to the reference values, moose immobilized with helicopter have significantly higher cortisol levels than moose immobilized from a car or on foot indicating that immobilizing from helicopter are most stressful, and moose immobilized with etorphine, have significantly higher cortisol levels than moose immobilized with medetomidine-ketamine, probably owing to a stronger drug response and secretion of cortisol, see figure 1. Both medetomidine-ketamine and etorphine significantly increase blood cortisol concentration. Arnemo (1995) compared immobilizing moose with medetomidine-ketamine with dart gun from a car or on foot, with moose immobilized with etorphine from helicopter. Several blood stress parameters were significantly lower in moose treated with medetomidine-ketamine than in animals treated with etorphine and might indicate a difference in the stress response of the drugs (Arnemo, 1995). In captive, semi-domestic reindeer immobilized with medetomidine-ketamine, cortisol levels increased two-to threefold during a 30-minute monitoring period, reaching mean levels of 220 to 300 nmol/L, and the increase started between 10 and 30

minutes after drug administration (Ryeng et al., 2001a; Ryeng et al., 2001b). Cockram et al. (2011) showed that plasma cortisol concentrations in red deer shot from helicopter were higher than deer shot by a single stalker or in a field, probably because the animal are more likely to be disturbed before it is shot. Helicopter makes noise, and noise is an important stressor that affects the welfare of captive laboratory animals (Carstens & Moberg, 2000). In the study of Lian (2012), moose were immobilized with a combination of etorphine, acepromazine and xylazine administered from helicopter, and with longer induction time than comparing studies. The cortisol concentration was not significantly higher than in the untreated, shot moose in this study, and may indicate the strong stress lowering effect of xylazine during decreasing of blood cortisol (Lian, 2012).

Even if some authors consider cortisol as a god stress indicator, others do not and some authors have been suggested that the measurement of several variables should give a more reliable estimate of the degree of stress (Morton et al., 1995). Catecholamines and corticosteroids released during the stress response have together with changes in hematological, serum biochemical and physiological parameters been used as indicators of stress in wild ungulates (DeNicola & Swihart, 1997; Mentaberre et al., 2010). Glucocorticoids convert stored glycogen in the liver and muscle to glucose, and in the absence of cortisol values, glucose levels have been used to evaluate stress indirectly (Stringer et al., 2011; Perez et al., 2005). Heart rate increases at capture due to both physical activity and handling the animal and catecholamines, and Mentaberre et al., (2010) suggest that heart rate variability (HRV) may be a better indicator of stress that cortisol. Generally physical capture and restraint can be very stressful for wild animals, methods must be developed to reduce the stress response, and administration of sedatives or anesthetics are recommended to prevent or reduce distress (Arnemo et al., 1993; Cattet et al., 2004; Oakley et al., 2004; Arnemo et al., 2005). Anyway, it is important to assess stress during capture and anesthesia of wild animals, but stress cannot be based on measurement of blood cortisol alone. In addition to measurement of selected blood constituents, it should be based on several variables including clinical examination (Arnemo & Caulkett, 2007).

On the basis of the findings in this study, the conclusions are: 1) Reference values of cortisol from chemical immobilized moose are influenced both of drugs and capture method. 2) Blood cortisol concentration from animals instantly killed by shooting should be used to establish baseline (“normal”) values of cortisol in this species. 3) Serum cortisol cannot be used as the only variable to assess acute stress in free-ranging moose.

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