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# URINARY CORTISOL AND UREA NITROGEN RESPONSES IN IRREVERSIBLY UNDERNOURISHED MULE DEER FAWNS

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ABSTRACT: We examined the concentration of urinary cortisol and urea nitrogen of five hand-reared mule deer (*Odocoileus hemionus*) fawns that failed to recover from winter starvation, and compared them to levels found in fawns that recovered. The fawns wintered in fenced pastures stocked with wild deer, and were put back on supplemental feed after losing 15% of their body mass. The five fawns that died began receiving supplemental feed up to 3 wk before death. All continued to lose weight, and were consequently removed from the pasture and fed ad libitum 4 to 10 days before death. In the animals that died, cortisol levels continued to increase regardless of food availability, and were correlated with those of urea nitrogen. Postmortem cortisol and urea nitrogen measurements were significantly greater than concentrations found in the weeks preceding death. We hypothesize that uncontrolled protein catabolism is promoted by high levels of cortisol. These cortisol levels may reach a point at which irreversible multiple-system organ failure occurs, leading to the animal's death.

*Key words:* Cortisol, urea nitrogen, starvation, irreversible undernourishment, organ failure, mule deer, *Odocoileus hemionus*.

## INTRODUCTION

Much time and effort have been devoted to emergency feeding programs for starving deer (Baker and Hobbs, 1985). Despite intensive efforts to nourish starving deer, in many cases reversal of the starvation process has failed (Cushwa et al., 1984). Short (1981) termed this phenomenon as a state of irreversible undernourishment.

The two explanations that have been offered for this phenomenon are (1) a starvation-induced reduction in the viability and digestive function of rumen bacteria (Nagy et al., 1967) and (2) adrenal exhaustion (Panaretto and Ferguson, 1969). DeCalesta et al. (1974) demonstrated an adequate population of rumen bacteria remained viable during long periods of starvation. The occurrence of high levels of blood cortisol maintained by wild-captured bighorn sheep (*Ovis canadensis canadensis*) over a period of 3 mo (Harlow et al., 1987) strongly suggests that adrenal exhaustion does not occur either. Another possible cause of irreversible undernourishment is multiple-system organ failure (MSOF) (Border et al., 1976). In human patients suffering from trauma, irreversible MSOF occurs because of a negative nitrogen balance (Border et al., 1976; Cerra, 1987). The negative nitrogen balance is a result of the patient's inability to utilize exogenous energy resources and increased catabolism of lean body tissue (Gusberg, 1986).

There are few data on whether irreversible MSOF occurs in starving animals. During starvation, catabolism of lean body tissue will be limited while fat reserves are still plentiful (Cahill, 1979). This muscle-sparing effect will be compromised once fat reserves are depleted (Moore, 1962; DeCalesta et al., 1975; Bahnak et al., 1979), or if starvation is accompanied by another stressor (e.g., cold weather) (Panaretto, 1968). Amino acids from the catabolized tissue will be used by the liver for energy, and will produce urea. In ruminants the urea can be recycled if carbohydrates are availabl⁻ (Robbins et al., 1974). If carbohydrates are limited, the urea will be excreted, resulting in a negative nitrogen balance and causing the degeneration of liver, kidney, and muscle tissue (Rothenbacker and Kradel, 1984), and possibly resulting in MSOF.

The catabolism of lean body tissue is



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induced by excessive concentrations of plasma cortisol (Klasing, 1985). Both urinary cortisol and urea nitrogen are strongly correlated to the plasma levels of these substances (Beisel et al., 1964; Warren et al., 1982; Miller, 1989). Thus, extreme postmortem levels of urinary cortisol accompanied by high levels of urea nitrogen would support the MSOF hypothesis rather than adrenal exhaustion. In this study, we contrast pre- and postmortem levels of urinary cortisol and urea nitrogen found in five mule deer (*Odocoileus hemionus*) fawns that died of irreversible winter starvation. We compared these levels with levels found in fawns that successfully recovered from starvation.

## METHODS

Data presented here are part of a larger study aimed at measuring mule deer fawn urinary cortisol and urea nitrogen responses to winter population density and environmental variables (Saltz, 1988). Hand-reared mule deer fawns were placed in two large pastures (66 and 169 ha) stocked with wild deer (39°59.5'N, 108°10'W). The deer spent the winter in these pastures feeding on natural vegetation. The pastures were stocked with wild deer at high (133 deer/km$^2$) and low (44 deer/km$^2$) densities, respectively. We collected weekly urine samples between 28 December 1986 and 14 April 1987 by placing the fawns in individual urine collecting cages located in the pastures. Fawns remained in the cages an average of 5.8 ± 1.6 hr. Study area description and urine collecting methods are detailed in Saltz (1988).

Fawns were weighed weekly. To minimize risk of mortality from starvation, fawns that lost 15% of their maximum recorded body mass began receiving supplemental feed (Baker and Hobbs, 1985) of 0.9 kg/day. The ration contained at least 21% crude protein, 3.5% fat, 0.05% iodine, and 42% neutral detergent fiber. The ration provides 2.55 kcal/g metabolizable energy, and at 0.9 kg/day would supply enough energy for maintenance (Cowan and Clark, 1977). If weight loss continued, the fawns were moved to the pens in which they were reared and fed ad libitum. Urine collection continued on a weekly basis in the pens using a long pole with a cup attached to the end of it. Postmortem samples were obtained within 24 hr after death by dissecting the animal and extracting the urine from the bladder with a syringe.

Urine cortisol will remain intact at room temperature (22 C) up to 36 hr and while frozen (−20 C) 2 to 3 yr (M. Miller, pers. comm.). Samples collected were placed in coolers containing snow and frozen upon return to the laboratory on the day of collection. Analysis for cortisol was done at the Endocrine Laboratory, Department of Physiology, Colorado State University (Fort Collins, Colorado 80523, USA) using radioimmunoassay procedures described by Reimers et al. (1981). Analyses for urea nitrogen and creatinine were performed by the Clinical Pathology Laboratory, Department of Clinical Sciences, Colorado State University (Fort Collins, Colorado 80523, USA) using urease and Jaffe (1886) procedures, respectively, on a Beckman analyzer (Beckman Inc., Fullerton, California 92634, USA).

The cortisol radioimmunoassay procedures were validated using two procedures, parallelism and quantitative recovery (Harlow et al., 1987). To test for parallelism, an 8-point 2.5-fold dilution series from a concentration of 0.16 to 100 ng/ml was devised to generate a standard curve using 100 μl of stock cortisol solution (100 ng/ml). Three similar curves were created from 3 pools of urine known to contain high, medium, and low levels of cortisol, respectively. The curves were generated using 200 μl of samples in five-step, two-fold dilution series. Two replicate series were constructed for each sample medium. Aliquots from the standard and urine were extracted and assayed following Olson et al. (1981). The logit transformation of percent buffer control bound was regressed by method of least squares on the log of the sample amount assayed. Slopes of the different urine curves and the standard curve were tested for homogeneity of slopes (PROC GLM, SAS Institute Inc., 1987). The regression coefficients for the three urine pools were −2.15, −2.07, and −1.92 (low, medium, and high cortisol respectively). None were different ($P > 0.3$) from the −2.05 slope of the standard solution.

We examined quantitative recovery with a four point two-fold dilution from 5 to 40 ng/ml of cortisol added to urine samples from a pooled urine medium originally containing low levels of cortisol. Extraction and assay followed Olson et al. (1981). The amounts recovered were regressed by the method of least squares on the amounts added, and the hypothesis slope = 1.0 was tested. The regression coefficient was 1.09 ± 0.05, and was not different ($P > 0.1$) from 1.0 ($r^2 = 0.99$). Thus, cortisol added to the urine before extraction and assay was accurately recoverable.

All statistics were performed on and presented as μg cortisol:mg creatinine (Ct:C) and mg

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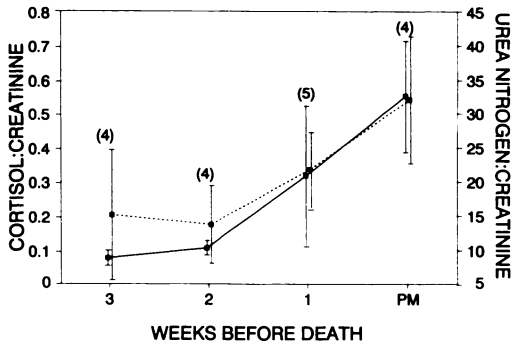



FIGURE 1. Mean (±2 SE) cortisol : creatinine (——) ratios and urea nitrogen : creatinine (- - -) ratios in urine samples collected from five re-fed undernourished mule deer fawns during 3 wk preceding their death, and samples collected postmortem (PM). Numbers over bars are sample size.

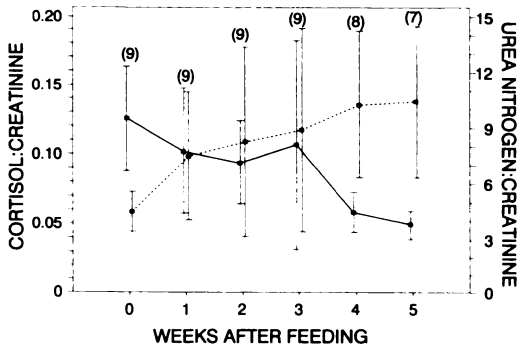



FIGURE 2. Mean (±2 SE) cortisol : creatinine (——) ratios and urea nitrogen : creatinine (- - -) ratios in urine samples collected from undernourished mule deer fawns that consequently survived, immediately before and up to 5 wk after re-feeding began. Numbers over bars are sample size.

urea nitrogen:mg creatinine (UN:C). Comparison of Ct:C and UN:C values among urine samples collected at different times was done by analysis of variance for unbalanced designs (PROC GLM, SAS Institute Inc., 1987), and Student's $t$-test. Effects were considered significant at $P < 0.05$.

## RESULTS

Sixteen fawns placed in the pastures began receiving supplemental feed after losing 15% of their maximum recorded body mass. Ten of these fawns continued losing weight and were moved back to the pens. Five of the 10 fawns died 4 to 10 days later, up to 3 wk after they began receiving supplemental feed. Postmortem urine samples were collected from four of the five fawns. Ct:C values increased rapidly during the last few weeks before the fawns died (Fig. 1). Mean postmortem Ct:C value was 0.550 ± 0.081 (SE), and was higher ($P < 0.05$, Tukey's Studentized Range Test) than values found 2 and 3 wk before death occurred (0.106 ± 0.010 and 0.077 ± 0.011). One wk before death Ct:C values were intermediate (0.317 ± 0.102), and were not different from either the postmortem samples or those taken 2 and 3 wk before death. By comparison, mean summer Ct:C value of 15 hand-reared fawns receiving an ad libitum diet was 0.011 ± 0.002, and was lower ($P < 0.001$) than postmortem values.

Mean postmortem UN:C value was 32.0 ± 4.6, and was higher ($P < 0.05$, Tukey's Studentized Range Test) than values found 2 and 3 wk before death (13.7 ± 2.8 and 15.1 ± 4.7). By comparison, mean summer values of urinary urea nitrogen of 15 hand-reared fawns receiving an ad libitum high protein diet was 17.2 ± 0.5, and was lower ($P < 0.05$) than postmortem values (Saltz, 1988).

In contrast to the fawns that died, Ct:C decreased in the supplementally fed fawns that survived (Fig. 2). Over the remainder of the winter the decrease was significant ($P < 0.01$), and was influenced by weather conditions and the density of the deer in the pastures (Saltz, 1988). Mean Ct:C in these fawns immediately before supplemental feeding began was 0.126 ± 0.019 and was lower, although not significantly ($P > 0.05$) than values in animals that died (0.297 ± 0.108, $n = 4$). UN:C increased ($P < 0.01$) (Saltz, 1988) in surviving animals after feeding began (Fig. 2), but after 5 wk of feeding was still lower ($P < 0.02$) than UN:C in postmortem samples. Immediately before feeding began, mean UN:C in fawns that died was 13.4 ± 3.2 and was higher, although not significantly

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($P > 0.05$) than UN:C value in the fawns that survived (4.52 ± 0.549).

## DISCUSSION

Although confinement may induce stress, cortisol levels in domestic sheep (Berman et al., 1980) and hand-reared Rocky Mountain bighorn sheep (Miller, 1988) remained unchanged during short term confinement (<12 hr). We found no correlation between length of confinement and Ct:C values (Saltz, 1988). Hence, it is assumed the observed stress responses in this study are free of any confinement effects.

Coupled with the available vegetation in the pastures, the supplemental feed should have provided enough energy for the fawns to survive and recover after losing 15% of their weight. Nevertheless, 10 fawns continued to lose weight (an additional 1% to 4%), and five died 4 to 10 days after ad libitum feeding began. While Ct:C in the surviving animals gradually declined after feeding began, values continued to increase in the animals that died. Extreme values of Ct:C found in urine taken from dead fawns were associated with high values of UN:C, suggesting that adrenal exhaustion did not occur. Moreover, UN:C levels exceeded those found in supplementally fed fawns that survived, as well as fawns being fed ad libitum during the summer. Extreme values of UN:C associated with irreversible undernourishment in deer were also described by DelGiudice and Seal (1988). This reflects the catabolic effect cortisol has on lean-body tissue, and supports the MSOF hypothesis. If organ failure is the cause of death in irreversible undernourishment, several questions arise. Does organ failure occur early and is it irreversible? Or, does some other irreversible process, causing lean body tissue catabolism, induce eventual organ failure and therefore death?

We hypothesize that extreme concentrations of cortisol induce irreversible responses in undernourished animals. Although protein synthesis responds mainly to substrate availability, net protein breakdown appears to be obligatory to the level of cortisol (Moyer et al., 1981; Powell-Tuck et al., 1984). This occurs via suppressed protein synthesis rather than increased proteolysis (Odedra and Millward, 1982). Because body protein is continually broken down and replaced, the inhibition of protein synthesis causes a net breakdown of body protein. Consequently, muscle wasting results from excessive cortisol concentrations, regardless of the amount and quality of food ingested by the animal, or blood insulin levels (Powell-Tuck et al., 1984). Therefore, severely undernourished deer may reach a point at which cortisol has attained concentrations where protein synthesis, even under optimal feeding conditions, will be suppressed beyond the point of being able to compensate for the loss of lean body mass; in other words, condemning the animal to starvation with a full stomach.

Branched-chain amino acids seem to have a marked effect on protein synthesis (Fulks et al., 1975). Border et al. (1976), Cerra et al. (1987), and Moyer et al. (1981) have demonstrated that branched-chain amino acids are capable of bypassing the inhibitory effects of cortisol. Thus, Cerra et al. (1987) suggest that under severe stress a high intake of branched-chain amino acids may be necessary to attain equilibrium with their loss resulting from catabolism. Several researchers have documented better results in refeeding starving deer when using high-protein diets as compared to high-energy diets (Cook and Harris, 1968; DeCalesta et al., 1975). Further research should be directed at determining the effect of diets with a high content of branched-chain amino acids on the consequent survival of refed undernourished deer.

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