Dehydration in stressed ruminants may be the result of a cortisol-induced diuresis


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ABSTRACT: The effect on water and electrolyte balance of stress, simulated by intravenous infusion of cortisol, was studied using 24 18-mo-old Merino wethers (37.0 ± 0.94 kg mean body weight [BW]) over 72 h. The sheep were allocated to one of four groups: 1) no water/no cortisol (n = 6); 2) water/no cortisol (n = 4); 3) no water/cortisol (n = 6); and 4) water/cortisol (n = 4). Animals allocated to the two cortisol groups were given 0.1 mg·kg BW	extsuperscript{−1}·h	extsuperscript{−1} of hydrocortisone suspended in isotonic saline to simulate stress for the duration of the experiment. Total body water, plasma cortisol, osmolality and electrolytes, and urine electrolytes were determined at 24-h intervals for 72 h. In the presence of cortisol, total body water was maintained in the face of a water deprivation insult for 72 h. Water deprivation alone did not induce elevated plasma concentrations of cortisol, in spite of a 13% loss of total body water between 48 and 72 h. Infusion of cortisol was found to increase urine output ($P = 0.003$) and decrease total urinary sodium output ($P = 0.032$), but had no effect on plasma electrolyte levels or water intake. Water deprivation was found to increase plasma sodium concentrations ($P = 0.037$). These results indicate that sheep given cortisol to simulate stress suffer from a loss of body water in excess of that associated with a loss of electrolytes, and support the hypothesis that elevated physiological concentrations of cortisol induce a diuresis in ruminants that contributes to dehydration.

Key Words: Body Water, Electrolytes, Hydrocortisone, Sheep, Stress

Introduction

Researchers have endeavored to discover the physiological changes that occur when animals are exposed to stressors by utilizing models that mimic the effects of the hypothalamo-pituitary-adrenal (HPA) axis. The HPA axis, when activated by stressors such as transport and handling, responds with the release of glucocorticoids and other hormones that have physiological effects. Cortisol is the principle stress hormone associated with the activation of the HPA axis, and it has been shown to induce pathophysiological changes to the immune (Roth and Kaeberle, 1982), metabolic (Sapolsky et al., 2000), and reproductive (Macfarlane et al., 2000) systems of animals.

Swanson and Morrow-Tesch (2001) highlighted the need for a valid model system to evaluate the physiological effects of transport stress in ruminants. Lay et al. (1996) proposed a stress response model based on an adrenocorticotropic hormone challenge test, but failed to accurately predict physiological disturbances seen in cattle subjected to transport and handling stress. Other authors have used glucocorticoids; Anderson et al. (1999) used dexamethasone to quantify the effects of potential stressors on immune competence in ruminants, and Macfarlane et al. (2000) utilized stress-like infusions of cortisol to model reproductive responses to stressors in Merino sheep.

The application of the Macfarlane et al. (2000) model to test the effects of stress-like infusions of cortisol on water and electrolyte balance has yet to be investigated. Thus, this study was conducted to test the hypothesis that elevated plasma concentrations of cortisol induce a diuresis that contributes to water loss in excess of electrolyte loss in Merino sheep.

Materials and Methods

Animals and Management

All experimental procedures were reviewed and approved by the animal ethics committee at James Cook University.
University (approval No. A664-01). Twenty-four 18-mo-
old Merino wethers (37.0 ± 0.94 kg mean BW) were
sorted in ascending order of BW, allocated to metabo-
lism crates at random, and fed oaten chaff ad libitum
for 10 d prior to the commencement of the experiment.
Upon entry to the crates, all animals were dosed with
Ivermectin (Ivomec-RV, 1 mL/10 kg BW; Merial Austra-
lia Pty Ltd., Parramatta, NSW, Australia) and their
necks and pizzels were shaved. The mean daily temper-
ature-humidity indices (THI) during the experimental
period for d 0, 1, 2, and 3 were 76, 76, 77, and 77,
respectively. There was no significant difference be-
tween THI for the acclimatization period or the experi-
ment.

Sample Collection

On d 0, 10 mL of blood was manually collected from all
treatment groups into tubes containing lithium heparin
(Disposable Products Pty Ltd., Adelaide, SA, Australia)
at 3-h intervals for 72 h. Intakes of water and feed were
measured daily. Total urine excreted was collected,
measured, and subsampled daily for 3 d during the
study. Urine samples were stored at −20°C until they
were analyzed. Blood samples were immediately placed
into an ice-water slurry, centrifuged at 200 × g for 15
min, and plasma was poured off within 2 h and frozen
(−20°C) for analysis at a later date. Plasma cortisol
concentration was measured using a RIA kit (Spectria
Cortisol 125I-coated tube kit, Orion Corp., Espoo,
Finland).

Urea Space Measurements

Urea space was determined on d 0, 1, 2, and 3 for
each animal using the technique described by Preston
and Kock (1973). In brief, following catheterization of
the jugular vein, a solution containing 20% (wt/vol) urea
dissolved in 0.9% (wt/vol) saline was administered
through the catheter over a 2-min period. The volume
injected was calculated to provide 130 mg of urea/kg
live weight. The catheter was flushed with 10 mL of
isotonic saline followed immediately by 10 mL of hep-a-
rinized saline solution (35,000 IU/mL of 0.9% saline)
to prevent clotting between samplings. Blood samples
were collected through the catheter prior to infusion
and at 15 min postinfusion. The following formula was
used to calculate urea space as a percentage of live
weight (Kock and Preston, 1979):

\[
\text{Urea space (\%)} = \frac{\text{[volume infused (mL)] × concentration of solution (mg urea nitrogen/dL)]/[plasma urea nitrogen/live weight (kg)]}}{\text{[volume infused (mL)]}}
\]

Treatments

Crate numbers were assigned at random, in a 2 × 2
factorial arrangement, to one of four groups: 1) no wa-
ter/no cortisol (n = 6); 2) water/no cortisol (n = 4); 3) no
water/cortisol (n = 6); and 4) water/cortisol (n = 4). On
d 0, all animals were catheterized with a polyvinyl chlo-
ride tube (o.d. 2.0 mm × i.d. 1.0 mm; Critchley Electrical
Products Pty Ltd., Silverwater, NSW, Australia) in-
serted into the jugular vein under local anesthetic.
Urine collectors were also fitted to the animals. All
animals allocated to the two cortisol groups were given
0.1 mg·kg BW−1·h−1 of hydrocortisone (Solu-Cortef, Up-
john Pty Ltd., Rydalmere, NSW, Australia) suspen-
ded in isotonic saline, administered at a rate of 0.1 mL·kg
BW−1·h−1, to simulate stress for the duration of the ex-
periment, as per Macfarlane et al. (2000). The noncorti-
sol groups were given an equivalent placebo infusion
of isotonic saline. Animals that were in water-deprived
groups had their water withdrawn at the commenc-
ent of the experiment.

Statistical Analysis

A 2 × 2 factorial arrangement, with the main effects
for water (ad libitum water and no water) and cortisol
(cortisol infusion and no cortisol), and the interaction
effects of water × cortisol with time taken into account
were analyzed statistically with a repeated-measures
ANOVA using SPSS 10 software package (SPSS, Chi-
cago, IL) Quantitative variables (plasma and urinary
electrolytes, plasma cortisol, total body water, and
urine output) were independently sampled. Tests for
sphericity and homogeneity were conducted to test as-
sumptions for the repeated-measures ANOVA, and in
all cases, these tests were satisfied. Arithmetic means
and standard errors have been presented; multiple com-
parison tests within the factors were not performed
because there were fewer than three groups and there-
fore any difference would be clearly perceived. Differ-
ences were considered significant for P < 0.05. Four
animals had to be withdrawn from the experiment, one
for scours and three for blocked catheter lines.

Results and Discussion

Plasma Cortisol Concentration

Macfarlane et al. (2000) maintained plasma cortisol
concentration at 72.0 ± 2.5 ng/mL to simulate stress in
sheep. The infusion rate chosen in this study appears
to offer a physiological dose rate when mean plasma
cortisol concentrations (Figure 1) are compared to those
found in sheep exposed to the stress of isolation and
Figure 1. Plasma cortisol concentrations (mean ± SEM) at 0, 24, 48, and 72 h for four groups of sheep in which stress was simulated by injection of cortisol (●) or not (○), and which were either water deprived (dotted line) or given ad libitum access to water (solid line). restraint (70 ng/mL; Apple et al., 1993), shearing and shearing noise (78.8 and 58.1 ng/mL; Hargreaves and Hutson, 1990), and handling stress prior to slaughter (22.0 to 77.8 ng/mL; Pearson et al., 1977). Of note is the fact that water deprivation alone for 72 h in the no water/no cortisol group did not increase plasma cortisol concentration to the same levels reported by other authors (Pearson et al., 1977; Hargreaves and Hutson, 1990; Apple et al., 1993). Others have touted water deprivation as being a significant stressor in the marketing process for ruminants (Atkinson, 1992). The lack of cortisol response between the water/no cortisol and no water/no cortisol group may be due to the animals having been derived from a population in the seasonally dry tropics in which water deprivation for 72 h, to a well-hydrated animal with ample water in the gastrointestinal tract, may not be a significant stressor. Similarly, Finberg et al. (1978) found no significant change in plasma cortisol concentration throughout 8 d of water deprivation in the camel. It would appear that water deprivation alone for 72 h in merino sheep is not a prototypical stressor that will activate the HPA axis. However, a HPA axis response may be invoked at an increased time of water deprivation. Blair-West et al., (1972) demonstrated a significant increase in plasma cortisol concentrations in sheep after 9 d of water restriction.

Body Water

There was no change in body water (Figure 2) within any group at 0, 24, or 48 h. The no water/no cortisol group sustained body water at 24 and 48 h (52.2 ± 4.2% and 53.2 ± 5.1%, respectively), before losing 13% by 72 h (40.2% ± 5.7%). A time × water interaction demonstrated body water loss for the no water groups between 24 and 48 h (P = 0.034) and 48 and 72 h (P = 0.052) compared to the groups on ad libitum water. Preston and Kock (1973) concluded that urea space in the ruminant was a measure of empty body water (total body water less the water in the gastrointestinal tract). The lack of reduction in body water for the water/cortisol group in spite of the presence of a diuretic effect may be due to the replacement of water in the urea space of the animal with water from the gastrointestinal tract in a bid to maintain homeostasis in the face of a net water deficit.

Urine Output

There were cortisol × water × time (P = 0.037) and a cortisol × time (P = 0.003) interactions between 24 and 48 h, demonstrating an increase in urine output for the water/cortisol group over the other groups during the same period (Figure 3). This interaction was not significant at the 48- to 72-h interval for the water/cortisol group was a measure of empty body water (total body water less the water in the gastrointestinal tract). The lack of reduction in body water for the water/cortisol group in spite of the presence of a diuretic effect may be due to the replacement of water in the urea space of the animal with water from the gastrointestinal tract in a bid to maintain homeostasis in the face of a net water deficit.

Figure 2. Empty body water (mean ± SEM) at 0, 24, 48, and 72 h for two groups of sheep that were either water deprived (dotted line) or given ad libitum access to water (solid line).

Figure 3. Total urine output (mean ± SEM) at 24, 48, and 72 h for four groups of sheep in which stress was simulated by injection of cortisol (●) or not (○), and which were either water deprived (dotted line) or given ad libitum access to water (solid line).
group, although a trend ($P = 0.07$) toward increased urine output continued for this group. One of the proposed avenues of weight loss in domestic animals placed under stress is an increase in urination (El Nouty et al., 1977; Hutcheson and Cole, 1986; Kenny and Tarrant, 1987; Phillips et al., 1991; Knowles, 1999), and it is believed that this increase in urination contributes to dehydration in the animal when water is unavailable (Phillips et al., 1991; Atkinson, 1992).

The mechanism by which cortisol induces a diuresis is still under debate. Pharmacological doses of cortisol in the dog (Baas et al., 1984) have been reported to induce a polyuria via inhibition in the action of the antiuretic hormone, arginine vasopressin (AVP). El-Nouty et al. (1977) demonstrated a significant increase in AVP concentrations in cattle during heat stress over thermoneutral conditions. The increase in AVP in heat-stressed cows was not associated with significant changes in urine output or glucocorticoid concentrations. The failure of El-Nouty et al. (1977) to detect changes in glucocorticoids to heat stress may lie in their sampling regimen. Cattle subjected to heat stress display rapid increases in plasma corticoid concentration followed by a decline (Lee et al., 1975). El-Nouty et al. (1977) sampled the animals after 2 d of heat stress when the cows may have adapted to the stressor. Short-term isolation stress in sheep by Parrott et al. (1987) invoked a similar trend toward a negative relationship between cortisol and AVP. High plasma cortisol concentrations were associated with low plasma AVP concentrations.

Glucocorticoids inhibit the vasoconstrictive and water-retentive effects of AVP by increasing the glomerular filtration rate (Wintour et al., 1985) and increasing the secretion and efficacy of atrial natriuretic peptide, both of which enhance water excretion. This mechanism has been suggested to prevent an overshoot by the vasoconstrictive effects of AVP (Sapolsky et al., 2000). This response may explain why the greatest contributing factor to the two- and three-way interaction involving cortisol seen in the present study was the water/cortisol group, which showed the greatest increase in urine output at 24, 48, and 72 h, whereas the no water/cortisol group appeared to stabilize its urinary output at 24, 48, and 72 h. This suggests that stress-like concentrations of cortisol will induce a diuresis if water is available in a bid to prevent hypervolemia, and in the absence of water will protect water balance by decreasing urine output. The diuresis could not be explained by polydipsia since both watered groups increased their water intake from 24 to 48 h. However, it is likely in this case, in the presence of ad libitum water, that glucocorticoids promoted a diuresis by increasing the glomerular filtration rate (Rang and Dale, 1991).

El-Nouty et al. (1980) demonstrated a significant decrease in aldosterone concentrations during heat stress in cattle and considered this to be the main factor resulting in the polyuria associated with heat stress. It has been known for some time that repeated treatment with ACTH or glucocorticoids results in a diminished response of the glomerulosa zone of the adrenal gland in a number of species (Coghlan et al., 1979). Coghlan et al. (1979) demonstrated that prolonged ACTH treatment in sheep significantly reduced the aldosterone response to known stimulating vectors, including angiotensin II and salt depletion. Sustained stimulation of the HPA axis, as may occur in acute stress, has quite different effects on mineralocorticoids and glucocorticoids.

Stressor stimulation results in the aldosterone response decreasing to normal or even low concentrations within 24 h, whereas cortisol and other glucocorticoid secretions are well maintained. In contrast to the suppressive effects of excessive stimulation of the HPA axis on aldosterone secretion, other aldosterone secretagogues (angiotensin II and plasma potassium) have specific actions on the adrenal glomerulosa alone, and do not stimulate glucocorticoids. This perhaps explains the sustained and high levels of aldosterone associated with hypovolemic stress, where the rennin-angiotensin system is the driving force (Espiner, 1987). Although elevated concentrations of cortisol in well-hydrated animals induces a diuresis, it would seem from the results of the present study that the principle effect of cortisol on the ruminant body is to protect and maintain water balance in times of stress.

**Water and Feed Intake**

High cortisol concentrations associated with stress have been noted to reduce and, in some sheep, cause complete abstinence from drinking (Guerrini and Bertiinger, 1982; Parrott et al., 1987). The cortisol/water group failed to repeat the responses observed by Guerrini and Bertiinger (1982) and Parrott et al. (1987), but demonstrated a time effect, increasing water intake between 24 and 48 h ($P = 0.001$) along with the no cortisol/water group (Table 1). There was also a time effect for decreasing feed intake between 48 and 72 h for all groups ($P < 0.001$).

Although mean daily THI increased by 1 unit at 48 h, it is doubtful that this would have had a significant effect on water or feed intake. In support of a lack of effect of THI on water and feed intake, the THI dropped below 74 for 9 h/d, allowing nighttime relief, and wind speed remained relatively constant at 9 km/h throughout the adaptation and experimental periods. In addition, throughout the 10-d adaptation period, the animals appeared to settle into their environment and were calm in the presence of the experimenters.

**Urinary Electrolytes**

A cortisol × time interaction for total sodium output ($P = 0.032$) between 24 and 48 h indicated that cortisol treatment resulted in a lower total daily sodium output in the urine of treated sheep than in untreated animals (Figure 4). There were no differences between groups at 72 h for total urinary sodium output.
Table 1. Water and feed intake by the four treatment groups of sheep at 24, 48, and 72 h after stress was simulated by injection of cortisol

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time, h</th>
<th>No water/no cortisol&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Water/no cortisol&lt;sup&gt;b&lt;/sup&gt;</th>
<th>No water/cortisol&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Water/cortisol&lt;sup&gt;b&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>Water intake, kg/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td></td>
<td>2.08 ± 0.49</td>
<td>2.45 ± 0.44</td>
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<tr>
<td>48</td>
<td></td>
<td>2.84 ± 0.31</td>
<td>3.01 ± 0.28</td>
<td></td>
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</tr>
<tr>
<td>72</td>
<td></td>
<td>2.58 ± 0.28</td>
<td>2.49 ± 0.25</td>
<td></td>
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</tr>
<tr>
<td>Feed intake, kg/d, as fed</td>
<td></td>
<td>0.69 ± 0.13</td>
<td>0.86 ± 0.14</td>
<td>0.67 ± 0.14</td>
<td>0.86 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>0.49 ± 0.05</td>
<td>0.73 ± 0.59</td>
<td>0.48 ± 0.59</td>
<td>0.83 ± 0.59</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>0.23 ± 0.82</td>
<td>0.45 ± 0.09</td>
<td>0.28 ± 0.90</td>
<td>0.59 ± 0.09</td>
</tr>
</tbody>
</table>

<sup>a</sup><sub>n = 6</sub>.  
<sup>b</sup><sub>n = 4</sub>.

Dehydration has been reported to induce a natriuresis in sheep and other species, including cattle (Bianca et al., 1965; McKinley et al., 1983; Metzler et al., 1986). This increase in sodium content in the urine of dehydrated animals is a homeostatic mechanism that allows sodium balance in the body to be maintained. Our studies demonstrate that although a natriuresis occurs with water deprivation (no water/no cortisol group), total urinary sodium content excreted per day actually decreases with total urinary volume as dehydration ensues. Studies that have evacuated the bladder of animals post-transport (Schaefer et al., 1992) merely illustrate that animal’s natriuretic mechanism due to water deprivation at that point in time. To extrapolate these results to promote the use of electrolyte solutions containing sodium in minimizing stressors is physiologically unsound.

There was a significant water × time interaction (\(P = 0.044\)) for total daily potassium output between 48 and 72 h, demonstrating an increase in potassium output with animals given access to water. Urinary potassium output tended to follow a similar trend to daily urine volume output. Water deprivation decreases the glomerular filtration rate of the kidney and, as such, less potassium would be excreted in urine compared to an animal offered ad libitum water. A time effect was significant between 24 and 48 h for the water/cortisol group (\(P = 0.041\)) (Figure 5), suggesting an increase in daily potassium output over the other groups. The time effect may be a reflection of the decreased feed intake experienced by the other groups. However, cortisol causes a degree of potassium loss through two pathways: 1) high physiological concentrations of cortisol can occupy mineralocorticoid receptors and induce mineralocorticoid activity (Rang and Dale, 1991); and 2) cortisol has been reported to increase the glomerular filtration rate promoting diuresis (Wintour et al., 1985; Rang and Dale, 1991).

Figure 4. Total urine sodium output (mean ± SEM) at 24, 48, and 72 h for two groups of sheep in which stress was simulated by injection of cortisol (solid line) or not (dotted line).

Figure 5. Total urine potassium output (mean ± SEM) at 24, 48, and 72 h for two groups of sheep that were either water deprived (dotted line) or given ad libitum access to water (solid line).
that were water deprived excreted less magnesium in their daily urine output than did animals that had ad libitum access to water (Figure 6). A time effect was significant \( (P = 0.042) \) at 48 h for the water/cortisol group, which had a higher level of daily magnesium excreted in urine over the other groups. The actions of calcitriotropic hormones are similar for calcium and magnesium and are said to influence magnesium in the kidney, affecting reabsorption (Saris et al., 2000). The action of cortisol is said to induce a negative calcium balance by decreasing calcium absorption in the gastrointestinal tract and increasing its excretion by the kidney (Rang and Dale, 1991). Although a trend continued for urinary magnesium loss in the water/cortisol group, plasma magnesium was unaffected. Hypomagnesemia in newly arrived feedlot sheep occurs within 10 d of arrival and is often associated with an increase in water consumption and loss of appetite (Franklin and Macgregor, 1944; Lucas, 1983). The increased water load must be excreted, and if outflow of magnesium exceeds inflow, hypomagnesemia occurs (Martens and Schweigel, 2000). Simulated stress via cortisol infusion failed to have any influence on plasma magnesium concentrations over 72 h.

**Plasma Electrolytes**

Plasma sodium concentrations had a significant water \( \times \) time interaction between 24 and 48 h \( (P = 0.037) \), indicating that water-deprived animals had a higher plasma sodium concentration than animals that had access to water (Figure 7). This trend was maintained throughout the rest of the study. Despite any mineralocorticoid effect cortisol may have had on sodium retention, water deprivation caused a greater increase in plasma sodium.

There was a trend toward a time \( \times \) cortisol interaction on plasma potassium concentrations from 0 to 24 h \( (P = 0.078) \) (Figure 8), indicating a lower plasma potassium concentration in cortisol-treated animals than animals that received no cortisol. Plasma potassium concentrations for all groups were less than the reported normal values for blood chemistry in sheep (4.8 to 5.9 mmol/L; Blood and Radostits, 1989). No clinical signs of potassium deficiency were detected in the experimental sheep or their flock mates.

Plasma magnesium concentrations were not affected by water deprivation or cortisol treatment. Cortisol treatment had no significant effect on plasma sodium,
potassium, or magnesium concentrations. Infusion of cortisol by Fan et al. (1975) into sheep has resulted in a similar outcome to that seen in the present study. Furthermore, these results are supported by other authors, who demonstrated that isolation and restraint stress in sheep had no effect on plasma sodium or potassium concentrations (Parrott et al., 1987; Apple et al., 1993).

Cole (2000) also demonstrated that feed and water deprivation for 72 h had no effect on plasma or whole blood sodium, potassium, or magnesium concentrations compared with hydrated, fed control sheep. Similarly, in other ruminants, Galyean et al. (1981) demonstrated no difference in plasma sodium concentration compared with unstressed controls, in steers subjected to fasting or transportation and fasting stress. In their study, Galyean et al. (1981) did, however, demonstrate a difference (P = 0.05) between plasma potassium concentrations at one sample point only (18 h) between the fasted and transported animals and control animals.

In stress-related research, the measurement of single variables (i.e., cortisol) is of little value when not considered in the context in which the substance is released, and when the consequences a particular level of the variable has for an animal's well being are not known (Von Borell, 2001). We concur with Parrott et al. (1987) that acute stress may activate a mechanism that enables the volume, tonicity, and ionic composition of the extracellular fluid in the sheep to be maintained in the face of a severe reduction in water intake. Cortisol seems to play a major role in activating this protective mechanism for the animal.

Implications

We would conclude from this model based on cortisol infusion that well-hydrated ruminants placed under stressful conditions will respond with a diuresis. However, because the animal may draw upon water reserves within its gastrointestinal tract, 72 h of cortisol infusion was not sufficient to observe a significant decrease in body water in the cortisol treated animals. As animals subjected to intravenous infusions of cortisol to stimulate stress seem to suffer from a loss of water in excess of that associated with a loss of electrolytes, administration of water alone is likely to be the most effective treatment for these animals.

Literature Cited


