Effects of corticosterone administration on nitrogen excretion and nitrogen balance in adrenalectomized rats

Zhi Yun Quan and Mackenzie Walser

ABSTRACT Adrenalectomized rats were implanted with pellets containing corticosterone in proportions varying from 0% to 100%, plus cholesterol. Stable concentrations of plasma corticosterone resulted, varying from subnormal (A) to physiologic (B) to supraphysiologic (C). When food was ingested ad libitum, weight gain was maximal in B at plasma corticosterone concentrations of 0.14–0.20 μmol/L; weight loss occurred in C, despite higher food intake. Even when rats had constant limited food intake, weight gain and positive nitrogen balance were significantly reduced in A compared with B because fecal nitrogen rose significantly and the retention of absorbed nitrogen for growth decreased. In C, weight decreased despite constant intake, and nitrogen balance became negative because urinary nitrogen increased markedly. We conclude that glucocorticoid insufficiency reduces nitrogen balance by impairing intestinal absorption of dietary protein and the utilization of absorbed nitrogen, whereas glucocorticoid excess reduces nitrogen balance by augmenting urinary nitrogen despite constant nitrogen intake. Am J Clin Nutr 1992;55:695–700.

KEY WORDS Glucocorticoids, urea excretion, food intake, fecal nitrogen

Introduction

Although it has long been recognized that corticosteroid administration induces negative nitrogen balance in animals and in normal humans (1–3), and the rise in corticosteroid production associated with injury has been implicated in the associated nitrogen wastage, the effects of variations in corticosteroid concentrations within the physiological range on protein balance are not well defined. There is little or no information concerning the effects of glucocorticoid insufficiency on nitrogen balance.

Several studies in growing farm animals indicate that low physiological concentrations of corticosteroid production are associated with faster growth (4–10), but this has not been a consistent finding (6, 11). Whether this observation reflects the antagonistic action of glucocorticoids on insulin stimulation of net muscle protein synthesis or simply indicates that corticosteroids participate in the metabolic response to food restriction is not clear (12).

Akana et al (13) varied plasma corticosterone concentrations in adrenalectomized rats given saline to drink by implanting subcutaneous pellets containing varying proportions of corticosterone and cholesterol. Weight gain was greatest in rats with plasma corticosterone concentrations between 0.13 and 0.21 μmol/L, a range that they concluded (from these and other data) was physiologic. Food intake was not controlled or measured in these studies. On the other hand, Santidrian et al (14) found that adrenalectomized and pair-fed control rats grew at the same rate.

The purpose of the present study was to determine nitrogen excretion and nitrogen balance in adrenalectomized rats maintained at subnormal, normal, and supranormal concentrations of plasma corticosterone and fed ad libitum or a constant amount.

Materials and methods

Animals and their care

Male Sprague-Dawley rats weighing 200–230 g were acclimatized for ≥ 1 wk to a room with controlled temperature and humidity and a light-dark cycle of 14 h light and 10 h dark. They were allowed free access to water and a standard 14% protein rat diet. The National Research Council’s guide for the care and use of laboratory animals was followed.

To prepare corticosterone-cholesterol pellets corticosterone and cholesterol powder (Sigma Chemical Co, St Louis) were mixed in ratios varying from 6.25% to 100% corticosterone. Aliquot samples of these mixtures are melted in a small beaker and poured into small wells (6 mm diameter) drilled into a block of paraffin. After the pellets cooled and solidified, they were removed from the paraffin with a scalpel and gently chiseled to free any adhering wax. Each pellet weighed ≈100 mg. Zero corticosterone pellets were made of paraffin wax alone.

Bilateral adrenalectomy was carried out under pentobarbital anesthesia through a midline dorsal incision in the skin and then muscle-splitting incisions in both flanks just behind the last ribs. The adrenal glands were gently lifted to the opening of the incision and curved scissors were used to remove the glands along with a small amount of surrounding adipose tissue. Care was
taken not to touch the adrenal gland with the dissecting instruments. Pellets of ~100 mg wax or a fused mixture of corticosterone and cholesterol (6.25%, 12.5%, 25%, 50%, or 100% corticosterone by weight) were inserted subcutaneously ~2 cm into the skin incision at the time of adrenal surgery.

After surgery, rats were housed individually in hanging wire-mesh cages. A standard 14% protein rat diet was used. Intake was ad libitum in experiments 1 and 2 but was limited to 7 g/100 g body wt in experiment 3, this being approximately the lowest ad libitum intake in experiments 1 and 2. Body weight and food intake were measured every day. When spillage occurred, food lost under the cage was collected and added to the unconsumed total before the food was weighed. Nevertheless, we consider the food intake measurements to be only approximate. Fresh food was provided daily, shortly before dark. A few rats who failed to ingest >10% of their total 24-h intake during a post-surgery test were eliminated from further study. All rats were provided with 0.9% saline in place of drinking water.

Protocols

Four experiments were performed. The purpose of experiment 1 was to determine whether constant plasma concentrations of corticosterone could be achieved in adrenalectomized rats implanted with pellets of corticosterone. The purpose of experiment 2 was to ascertain the pattern of ad libitum food intake and growth in adrenalectomized rats replaced with corticosterone at various concentrations. In experiment 3, nitrogen balance was determined from measurements of intake and excreta on days 6 and 7 following adrenalectomy in rats given pellets of various corticosterone content and fed a constant limited food intake. The purpose of experiment 4 was to determine the growth rate of intact rats fed the same food intake as the adrenalectomized rats of experiment 3; sham surgery was not performed because we wished to compare these results with the plateau growth rate, after recovery from the effects of surgery, in operated rats.

Specific protocols were as follows.

**Experiment 1.** Eighteen rats were divided into three groups of six rats each. At the time of the adrenalectomy, pellets containing 12.2%, 25%, or 50% corticosterone were implanted. On days 5, 7, 10, and 14, blood samples were obtained for corticosterone assay from the tail vein under light ether anesthesia.

**Experiment 2.** Thirty rats were divided into six groups of five rats each. At the time of the adrenalectomy, pellets containing 0%, 6.25%, 12.5%, 25%, 50%, or 100% corticosterone were implanted. Food was provided ad libitum. Food intake and body weight were recorded for 14 d.

**Experiment 3.** Seventy-two male rats were divided into six groups of 12 each and weighed. Rats were adrenalectomized as above and implanted with pellets containing each of the same six proportions of corticosterone and cholesterol as in Experiment II. After surgery, 7 g/100 g body wt of a standard 14% protein rat diet was given daily. On days 6 and 7 following surgery, 24-h urine and feces were collected from half of the rats in each group. A terminal blood sample was obtained. Urine, feces, and plasma were stored at ~20 °C and analyzed within 1 wk.

**Experiment 4.** Six male rats were acclimatized as above and then fed 7 g/100 g body wt of the standard 14% protein diet daily. Weight was recorded for 9 d. Results for the last 7 d were averaged.

**Analyses**

Plasma corticosterone concentrations were measured by radioimmunoassay on heat-denatured plasma samples by using a kit (ICN Biomedicals, Costa Mesa, CA). One gram of feces with 2 mL water was stored at 4 °C overnight, homogenized completely, and diluted to 100 mL. Dietary, fecal, and urinary nitrogen were measured in an Antek 720 nitrogen detector (Antek Instruments, Houston).

In experiment 3, nitrogen intake was calculated as measured feed intake times its measured nitrogen content (3.24%). Nitrogen balance was calculated as nitrogen intake minus fecal plus urinary nitrogen and expressed per 100 g body wt.

All results are shown as mean ± S.D. Statistical analysis was performed by one-way analysis of variance and Duncan’s multiple-range test (15).

Results of measurements of urinary nitrogen, fecal nitrogen, and nitrogen balance as functions of plasma corticosterone concentration were plotted and a smooth line was drawn to fit the data, without the point necessarily falling on the line. This was done by a SAS option called INTERPOL-SM (16). This routine produces a cubic spline that minimizes a linear combination of the sum of squares of the residuals of fit and the integral of the square of the second derivative.

**Results**

Sequential plasma corticosterone concentrations in experiment 1 are shown in Table 1. By the 14th postoperative day, corticosterone concentrations began to fall. Consequently, pellets were replaced weekly in subsequent experiments. There were clear differences in plasma corticosterone concentrations among the groups (p < 0.01, ANOVA).

**TABLE 1**
Sequential plasma corticosterone levels*
In Table 2 are shown weight gain and approximate food intake as functions of pellet corticosterone concentration in rats fed ad libitum (experiment 2). Food intake was apparently higher in the rats with the highest corticosterone-replacement concentrations. On the first postoperative day, all rats lost weight, as expected (data not shown). During days 2–7 and days 8–14, weight gain was significantly greater in group 4 than in all other groups. Groups 3 and 5 were similar (though slightly but significantly different on days 2–7). Group 1 grew significantly more slowly. Group 6 lost weight.

In experiment 3, in which food intake was constant, weight gain also stabilized by ≈1 wk. Weight gain increased as a function of pellet corticosterone content as follows: 0% < 6.2% < 50% < 12.5% < 25%. Maximal growth occurred near the lower end of the physiological range (0.13–0.21 μmol/L, ref 13). Between 0.09 and 0.20 μmol/L, daily growth averaged 1.28 ± 0.11 g/100 g body wt; in intact rats fed the same limited intake (experiment 4), growth averaged 1.00 ± 0.08 g/100 g body wt/d, a significantly (P < 0.05) lower value.

Total urinary nitrogen excretion averaged over postoperative days 6 and 7 as a function of plasma corticosterone concentration in experiment 3 is depicted in Table 3 and Figure 1. Urinary nitrogen is lowest at physiological concentrations of corticosterone and below. Supraphysiological concentrations of corticosterone lead to a marked and significant increase in urinary nitrogen.

Fecal nitrogen in these same rats is shown in Table 3 and is depicted in Figure 1. Below the physiological range of corticosterone concentrations, fecal nitrogen is significantly increased, accounting for about half of the observed decrease in nitrogen balance.

Results of nitrogen balance measurements on postoperative days 6 and 7 are shown in Table 3 and Figure 3. In the physiological range of plasma corticosterone, nitrogen balance is maximally positive. At lower levels of hormone replacement, including none, balance is still positive but significantly less so. This change is attributable in part to increased fecal nitrogen and in part to reduced retention of absorbed nitrogen, as shown by the marked decrease in crude biological value.

### Discussion

The role of glucocorticoids in protein metabolism has been studied extensively, but this is evidently the first study to characterize nitrogen balance throughout a range of corticoid concentrations encompassing subnormal, normal, and supraphysiological concentrations. The profound effects we observed are clearly multiphasic and are not well conveyed in terms of conventional statistical tests. We therefore used curves fitted by computer as well as statistical analysis to describe our findings.

The results clearly show that nitrogen balance is maximal at physiological concentrations of corticosterone. Adrenocortical insufficiency decreases nitrogen balance in part by increasing fecal nitrogen, indicating an impairment of intestinal absorption of dietary protein, and in part by reducing the retention for growth of absorbed nitrogen. Corticosterone excess reduces nitrogen balance by increasing urinary nitrogen excretion. In another study (17) we showed that this latter response is attributable to accelerated protein breakdown relative to protein synthesis.

We have been unable to locate previous studies of nitrogen balance or fecal nitrogen in adrenocortical insufficiency. Early work indicated that adrenalectomy, followed by salt maintenance, does not markedly alter growth or net nitrogen metab-

### Table 2

<table>
<thead>
<tr>
<th>Group (pellet corticosterone)</th>
<th>Approximate daily food intake</th>
<th>Daily weight change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days 2–7</td>
<td>Days 8–14</td>
</tr>
<tr>
<td>------------------------------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>1 (0%)</td>
<td>6.2</td>
<td>6.6 ± 2.0\textsuperscript{d}</td>
</tr>
<tr>
<td>2 (6.2%)</td>
<td>6.9</td>
<td>7.7 ± 0.7\textsuperscript{ad}</td>
</tr>
<tr>
<td>3 (12.5%)</td>
<td>7.3</td>
<td>10.7 ± 1.2\textsuperscript{b}</td>
</tr>
<tr>
<td>4 (25%)</td>
<td>7.8</td>
<td>12.5 ± 0.4\textsuperscript{a}</td>
</tr>
<tr>
<td>5 (50%)</td>
<td>8.3</td>
<td>9.0 ± 1.0\textsuperscript{e}</td>
</tr>
<tr>
<td>6 (100%)</td>
<td>10.9</td>
<td>−3.4 ± 0.6\textsuperscript{c}</td>
</tr>
</tbody>
</table>

* X ± SD; n = 6. Means lacking common superscripts within a given column are significantly different (P < 0.05).

### Table 3

<table>
<thead>
<tr>
<th>Group (pellet corticosterone)</th>
<th>Plasma corticosterone</th>
<th>Intake</th>
<th>Urine</th>
<th>Fecal</th>
<th>Balance</th>
<th>Biological value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmol/L</td>
<td>mg</td>
<td>mg</td>
<td>mg</td>
<td>mg</td>
<td>mg/100 g body wt</td>
</tr>
<tr>
<td>1 (0%)</td>
<td>0.011 ± 0.007\textsuperscript{a}</td>
<td>378 ± 16\textsuperscript{a}</td>
<td>168 ± 17\textsuperscript{bc}</td>
<td>152 ± 14\textsuperscript{a}</td>
<td>58 ± 16\textsuperscript{d}</td>
<td>22 ± 6\textsuperscript{a}</td>
</tr>
<tr>
<td>2 (6.2%)</td>
<td>0.072 ± 0.025\textsuperscript{ad}</td>
<td>357 ± 15\textsuperscript{b}</td>
<td>155 ± 10\textsuperscript{c}</td>
<td>125 ± 19\textsuperscript{b}</td>
<td>77 ± 12\textsuperscript{ad}</td>
<td>29 ± 4\textsuperscript{a}</td>
</tr>
<tr>
<td>3 (12.5%)</td>
<td>0.097 ± 0.020\textsuperscript{bd}</td>
<td>370 ± 12\textsuperscript{ab}</td>
<td>160 ± 21\textsuperscript{b}</td>
<td>99 ± 18\textsuperscript{c}</td>
<td>111 ± 18\textsuperscript{ab}</td>
<td>39 ± 4\textsuperscript{b}</td>
</tr>
<tr>
<td>4 (25%)</td>
<td>0.161 ± 0.020\textsuperscript{c}</td>
<td>381 ± 4\textsuperscript{a}</td>
<td>158 ± 19\textsuperscript{c}</td>
<td>102 ± 6\textsuperscript{a}</td>
<td>121 ± 13\textsuperscript{a}</td>
<td>45 ± 6\textsuperscript{a}</td>
</tr>
<tr>
<td>5 (50%)</td>
<td>0.266 ± 0.041\textsuperscript{a}</td>
<td>369 ± 15\textsuperscript{ab}</td>
<td>181 ± 10\textsuperscript{b}</td>
<td>96 ± 19\textsuperscript{a}</td>
<td>93 ± 25\textsuperscript{b}</td>
<td>35 ± 9\textsuperscript{b}</td>
</tr>
<tr>
<td>6 (100%)</td>
<td>0.638 ± 0.121\textsuperscript{c}</td>
<td>369 ± 5\textsuperscript{ab}</td>
<td>293 ± 22\textsuperscript{a}</td>
<td>104 ± 16\textsuperscript{a}</td>
<td>−28 ± 14\textsuperscript{b}</td>
<td>−12 ± 6\textsuperscript{b}</td>
</tr>
</tbody>
</table>

* X ± SD; n = 5–6. Means lacking common superscripts in a given column differ significantly (P < 0.05).

† These values are means of 2 consecutive days.

‡ Balance/(intake − fecal).
olism in rats, but the rate of release of amino acids from peripheral tissues was reduced (18). The dual metabolic actions of glucocorticoids have been pointed out by others. For example, Freedman et al (19) gave adrenalectomized Zucker rats daily injections of hydrocortisone at various doses and noted biphasic responses of gain in body protein and gain of body fat, the former peaking at a dose one-twentieth that of the latter. The difference between those results and the present study’s may relate to the use of hydrocortisone instead of corticosterone by those researchers or to the stress induced by daily injections in adrenalectomized animals but not in sham-operated controls.

N. Levin, SF Akana, CS Ciacio, L. Jacobson, M. Goodman, and MF Dallman [unpublished observations cited in Dallman et al (20)] implanted corticosterone pellets or added corticosterone to the drinking fluid of adrenalectomized rats. Food intake 3-5 d postoperatively was subnormal with zero replacement and with 12.5% pellets, but was normal with 25% pellets; higher doses were not studied.

Devenport et al (21) used long-acting corticosterone pellets in adrenalectomized rats and observed that weight gain and feed efficiency peaked at a serum corticosterone concentration of \( \approx 0.17 \) \( \mu \text{mol/L} \); the ratio of carcass fat to carcass protein increased monotonically. From these observations and observations with other hormones, they concluded that two receptor types are involved. Evidence for two corticosteroid receptor types in brain was recently reviewed (22).

On the other hand, Tomas et al (23) observed no difference in weight gain of adrenalectomized rats given daily injections of corticosterone at various doses and pair-fed to untreated adrenalectomized animals, until high doses were given. In that case, 3-methylhistidine excretion increased.

Although it is widely accepted that glucocorticoid administration induces net protein catabolism, this has proven difficult to establish consistently in humans, and negative (24-27) as well as positive (1, 3, 24, 28-30) results have been reported in studies that used a variety of hormones, doses, and experimental protocols. For example, in one recent study (24), in which normal subjects received 140 cortisol \( \mu g \cdot \text{kg}^{-1} \cdot \text{h}^{-1} \) for 64 h, urinary urea excretion was augmented at 12-36 h but not at 36-60 h, even though plasma cortisol concentrations and leucine and phenylalanine appearance rates, measured isotopically, remained elevated—a discrepancy the authors could not explain. On the other hand, Gelfand et al (1) found a sustained increase in nitrogen excretion during a 3-d infusion of cortisol, epinephrine, and glucagon in obese subjects.

It is likely that some of the conflicting results reported in the literature reflect the multiphasic nature of the effects of glucocorticoids, seen in the Figures 1-3. Our results indicate that responses to corticosterone will be highly dependent on dose. Although our results and those of Akana et al (13) point to a narrow range of plasma corticosteroid concentrations as being optimal for the growth and therefore “normal,” these inferences refer to an average hormone concentration throughout the day. It is documented that corticosterone concentrations in the plasma of rats vary widely in a circadian (30) and perhaps a circaseptan (31) manner. According to Krieger (30), plasma corticosterone concentration in rats fed ad libitum varies throughout the day from 0.06 \( \mu \text{mol/L} \) to 0.69 \( \mu \text{mol/L} \). Thus, if 0.14-0.21 \( \mu \text{mol/L} \) is “normal” (13), plasma corticosterone is “abnormal” about two-thirds of the time. Whether nitrogen metabolism responds rapidly to changes in corticosterone is uncertain, but if it does,
nitrogen metabolism and breakdown must also vary widely throughout the day, according to our results. The well-known effect of meals on protein turnover (32) may be mediated, at least in part, by changes in glucocorticoid concentrations, because meals stimulate glucocorticoid production (33-37), particularly high-protein meals (36-39).

It would appear, in fact, that nitrogen balance and growth in animals with constant corticosterone concentrations in the range of 0.14-0.23 μmol/L should be more positive than in intact rats, whose corticosterone concentrations vary widely. This appears to be the case: intact rats fed the same limited diet (experiment 4) grew 22% slower than did adrenalectomized rats with plasma corticosterone concentrations between 0.09 and 0.20 μmol/L. These results suggest that diurnal fluctuations in corticosterone concentrations of the magnitude reported (30) exert mildly deleterious effects on growth in comparison with sustained concentrations of corticosterone at normal concentrations in adrenalectomized animals.

These considerations point up the prevailing uncertainty about the physiological role of normal variations in glucocorticoid production. Although this process has been studied extensively, its function has never been clarified and only rarely discussed. According to Kumar et al (40), diurnal fluctuations in corticosterone concentrations may modulate food intake and nutrient selection.

Clearly, rats with supraphysiologic concentrations of corticosterone do less well postoperatively in terms of weight gain and nitrogen balance than do rats with physiologic concentrations (Figs 1 and 3). It would be interesting to determine how adrenalectomized rats implanted with 25% corticosterone pellets differ from intact rats in their rates of nitrogen excretion and in their responses to various forms of stress.

Although these findings clarify the relationship between corticosterone concentrations and nitrogen metabolism when corticosterone concentrations vary from near zero to supraphysiologic, they do not shed light on the effects of variations within the unstressed, physiologic range of mean 24-h hormone concentrations. It is not clear from Figure 3 whether higher concentrations of corticosterone within the range 0.12-0.23 μmol/L are associated with less-positive nitrogen balance. Further observations of adrenalectomized animals maintained at a narrower range of hormone concentrations, spanning the unstressed range of mean 24-h physiologic concentrations only, would be of interest. It remains to be established that the relationship between growth and spontaneous glucocorticoid concentrations reported in some studies of farm animals, summarized in the Introduction, is explicable in terms of the relationships found herein.  

References

2. Long CNH, Katzin B, Fry EG. The adrenal cortex and carbohydrate metabolism. Endocrinology 1940;26:309-44.
27. Parsons W, Crispell KR, Ebbert A. Abnormalities in N15 excretion


