Effects of glucocorticoids on glutamine metabolism in skeletal muscle

FERDINAND MUHLBACHER, C. RAJA KAPADIA, MICHAEL F. COLPOYS, ROBERT J. SMITH, AND DOUGLAS W. WILMORE
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Muhlbacher, Ferdinand, C. Raja Kapadia, Michael F. Colpows, Robert J. Smith, and Douglas W. Wilmore. Effects of glucocorticoids on glutamine metabolism in skeletal muscle. Am. J. Physiol. 247 (Endocrinol. Metab. 10): E75–E83, 1984.—The effects of dexamethasone on nitrogen and amino acid metabolism in the dog were studied in order to gain insight into the role of glucocorticoids in accelerated proteolysis and altered metabolism of glutamine in catabolic illnesses. After dexamethasone administration at a dose of 0.44 mg·day⁻¹·kg⁻¹, nitrogen balance shifted from slightly positive (+0.126 g·N·day⁻¹·kg⁻¹) to markedly negative (−0.978 g·N·day⁻¹·kg⁻¹). This was associated with a 23% fall in total free amino acid nitrogen in skeletal muscle, with 80% of the decline accounted for by a decrease in glutamine. Plasma glutamine concentration decreased by 26%, although total plasma free amino acid nitrogen was unchanged because of a 49% increase in alanine. The alterations in intracellular and circulating levels of glutamine were not accompanied by measurable changes in glutamine synthetase or glutaminase activities in skeletal muscle. Hindquarter amino acid flux measurements demonstrated that the decline in intracellular glutamine concentration was associated with a marked increase in glutamine efflux from skeletal muscle. This occurred in spite of minimal changes in the intracellular/extracellular glutamine gradient. It is concluded that accelerated muscle glutamine release caused by glucocorticoids is a major contributor to the decreased glutamine levels in muscle that occur during critical illnesses.

skeletal muscle flux; glutamine synthetase; glutaminase

CRIITICAL ILLNESS, whether secondary to accidental injury, severe infection, burns (20), or diabetic ketoacidosis (4), is characterized by a loss of body protein. The metabolic response to these stress states includes accelerated ureagenesis and increased urinary nitrogen excretion, sometimes accompanied by increased nitrogen loss from the body through other routes (20). It is thought that the major source of the nitrogen is skeletal muscle protein because of the large quantity of nitrogen lost from the body plus the appearance of muscle atrophy and progressive weakness during prolonged catabolic states. This conclusion is supported by the observations that total body protein breakdown is accelerated in critically ill patients (13), the urinary excretion of biochemical markers reflecting skeletal muscle proteolysis is increased (27), and the efflux of amino acids from the extremities of injured (5) and diabetic (1) patients is accelerated.

In addition to alterations in protein turnover and amino acid flux between tissues, critical illnesses are accompanied by characteristic changes in the concentrations of free amino acids both in the circulation and in skeletal muscle (3). Plasma amino acid concentrations are generally lower than normal, and the total pool of skeletal muscle amino acids, measured in tissue obtained by percutaneous muscle biopsy, is also decreased. In both plasma and skeletal muscle, there is an especially marked decline in glutamine concentrations. The levels of glutamine, which is the most abundant amino acid in plasma and accounts for 60% of the total free amino acid pool in muscle (excluding taurine), decrease by up to 70% in critically ill patients (3). Although the mechanisms for the alterations in amino acid concentrations in skeletal muscle are unknown, it has been proposed that there may be 1) an energy deficit in skeletal muscle resulting in amino acid consumption to meet increased energy demands (24), 2) an alteration in amino acid transport across the skeletal muscle plasma membrane (3), or 3) an inhibition of intracellular glutamine formation (23).

Critical illnesses are accompanied by alterations in the secretion and levels of several hormones that may influence amino acid and protein metabolism. Insulin concentrations may be low, normal, or even elevated, in association with tissue insensitivity to insulin (8). In general, concentrations of the counterregulatory hormones glucagon, cortisol, growth hormone, and the catecholamines rise during acute illnesses and return to normal with resolution of the disease process (30). The purpose of this study was, first, to characterize in an intact animal the effects of exogenous glucocorticoids on nitrogen balance and amino acid concentrations in the circulation and in skeletal muscle. The responses to this single hormone were then compared with the changes that occur during critical illness. The second purpose of the study was to focus on the amino acid glutamine since it plays a central role in amino acid metabolism and is markedly altered during illness. The effects of glucocorticoids on skeletal muscle glutamine concentration, efflux, synthetic, and degradative enzyme activities, and transmembrane gradients were characterized.
METHODS

Preparation of experimental animals. Conditioned mongrel dogs of either sex, weighing between 20 and 40 kg, were obtained from a farm where they had been regularly exercised and screened for parasites and other infestations. All females were proven to be nonpregnant. While in our kennels, the animals were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHENV publ. no. NIH 78.23, revised 1978). The dogs were kenned individually in an environment of constant temperature (20°C) and 24-h light exposure. They were exercised for 2 h every morning, provided with water ad libitum, and given a single meal of Agway Respond 2000 dry dog chow (approximate calorie distribution no less than 21% protein and 19% fat; the remaining energy as carbohydrates) daily between 1:00 and 3:00 P.M.

On the day prior to surgery, all unconsumed food was removed at 5:00 P.M. At 7:30 the next morning the dog was walked for at least 20 min and then taken to the operating suite, where it was anesthetized with intravenous pentobarbital sodium (Abbott Laboratories, 30 mg/kg body wt). An endotracheal tube was placed, and the animal was allowed to breathe a mixture of approximately 60% O₂ and air spontaneously. An intravenous solution of 0.9% saline was started, and penicillin G (E. R. Squibb, Princeton, NJ, 600 mg) and cephalothin (Keflin, Eli Lilly, Indianapolis, IN, 1 g) were given intravenously. The dog was placed supine on the operating table, the abdomen and flanks were shaved, and the skin was cleansed with soap and water and then povidone-iodine (Betadine) preparatory solution (Clinipad, Guilford, CT). The dog was draped with sterile sheets, and the abdomen was explored via a vertical, infraumbilical incision. The right deep circumflex artery and vein and the median sacral artery were isolated by blunt retroperitoneal dissection. A specially prepared catheter, consisting of a 6-cm segment of polyethylene tubing (2.08 mm OD) coated with Silastic linked to a 2.8-mm-OD polyethylene catheter via a metal connector, was inserted 6 cm cranially into the aorta via the right deep circumflex artery. A similar catheter was inserted into the median sacral artery, with its tip positioned about 1 cm proximal to the bifurcation of the aorta and distal to the inferior mesenteric artery. A venous catheter was inserted into the inferior vena cava via the right circumflex vein and positioned distal to the renal veins. All catheters were secured and then exteriorized posterior to the peritoneum through stab wounds in the flank. The abdominal musculature was closed, and the animal was then turned onto its left side. The exteriorized catheters were plugged with blunt needles closed by intermittent injection ports (Jelco, Critikon, Tampa, FL), flushed with saline, filled with heparin (1,000 U/ml), and buried subcutaneously so that the distal injection port was positioned high on the flank, close to the vertebral column. This allowed access to the aorta and inferior vena cava by percutaneous puncture of the injection ports of the catheters. Two further intravenous doses of cephalothin (1 g) were given 3 and 24 h postoperatively via the venous catheter.

Recovery from the surgical procedure was followed by observing the general well-being of the animals and by monitoring rectal temperature, food intake, body weight, hematocrit, and wound healing. The catheters were flushed with saline and refilled with heparin at weekly intervals. The animals were allowed to recover for at least 14 days following operation and then were studied only if they met the following criteria: 1) normal food intake, 2) normal stools, 3) maintenance or gain in body weight, 4) rectal temperatures below 103°F (normal range 100–102.5°F), 5) hematocrits greater than 30%, 6) white blood cell counts below 16,000 cells/ml, and 7) clean, well-healed wounds. Over 90% of the animals prepared and followed in this manner were studied.

At the termination of the experiments, the animals were killed by the administration of an overdose of intravenous barbiturate anesthesia. All animals underwent postmortem examination with careful inspection of the catheter positions. The catheter tracts were dissected, and the subcutaneous pockets in the flank were opened and inspected for fluid or blood accumulation or for evidence of infection. The abdomen and thoracic cavities were also carefully examined. In all the animals studied, acceptable catheter position was verified, and no signs of infection were found.

Nitrogen balance. Four dogs were placed in metabolic cages that allowed the separate collection of urine and stool. After 5 days of acclimatization, daily food intake was fixed by giving a single meal of dog chow (25 g/kg body wt) at 9:00 A.M. Food not consumed by the following morning was weighed and discarded each day. Water was provided ad libitum. Urine was collected during 24-h periods from 8:00 A.M. to 8:00 A.M. in a vessel containing 2 ml of concentrated sulfuric acid. The 24-h urine volume was measured and an aliquot was frozen at −20°C. Stools were collected at 24-h intervals and weighed. Four-day collections were pooled and homogenized, and aliquots were stored frozen. The nitrogen content of urine, stool, and food was determined by the macro-Kjeldahl method (21). Daily nitrogen input, excretion, and balance were determined during a 4-day control period followed by a 7-day experimental period during which the dogs were given 0.44 mg/kg body wt dexamethasone (Elkins-Sinn, Cherry Hill, NJ) daily as a single intramuscular injection in the shoulder.

Dexamethasone time course. The effect of dexamethasone on the concentrations of amino acids in whole blood, plasma, and skeletal muscle was followed over a period of 14 days. At least 2 wk following catheter placement, eight dogs were randomly allocated to two groups, one receiving 0.44 mg/kg dexamethasone intramuscularly daily and the other receiving an equal volume of 0.9% saline. Blood and tissue samples were obtained on days 0, 2, 4, 6, 8, 10, and 14 of the study. To ensure that the dogs were postabsorptive at the time of sampling, all food was removed from the kennels at 5:00 P.M. on the day prior to sampling, and all samples were obtained between the hours of 8:00 and 11:00 A.M. At the time of
study the dogs were placed in a Pavlov stand, and 5 ml of arterial blood were withdrawn from the aortic catheter, 3 ml of which were immediately deproteinized with 10% perchloric acid (see details on processing of blood and muscle below). A 10-ml aliquot of heparin-treated blood from a cephalic vein was centrifuged, and 3 ml of the supernatant plasma were deproteinized with 10% perchloric acid. After rapid induction of general anesthesia with intravenous thiopental sodium (Abbott Laboratories, 5 mg/kg body wt), skeletal muscle biopsies were obtained by percutaneous needle biopsy of the vastus lateralis muscle using the technique of Bergström (6). The vastus lateralis muscle was selected because it is easily located percutaneously and has been shown to be composed of predominantly fast-oxidative-glycolytic and fast-glycolytic fibers in several species (2). Thus, its fiber composition is representative of the bulk of skeletal muscle. In preliminary studies in five control animals, no change in intracellular concentrations of glutamine, glutamate, tissue water, glutamine synthetase, or glutaminase occurred when samples were taken at multiple time points between 3 and 60 min following induction of anesthesia. Subsequent tissue samples were obtained in less than 10 min after administration of anesthesia.

Determination of skeletal muscle glutaminase and glutamine synthetase activities. Nine dogs received dexamethasone (0.44 mg/kg body wt im daily) to determine the effect of glucocorticoids on skeletal muscle enzyme activities. Biopsies of the vastus lateralis muscle weighing at least 500 mg were obtained either by multiple needle biopsies or by open biopsy during brief thiopental anesthesia (5 mg/kg), both before and 7 days after starting glucocorticoid treatment. Samples for the simultaneous measurement of blood and intracellular concentrations of glutamine and glutamate were also obtained. The muscle pieces were weighed, immersed in 10 ml of buffer (0.1 M KCl, 0.001 M EGTA, and 0.05 M Tris, pH 7.4), and minced. The buffer was then drained and the muscle was resuspended in 5 ml of the same buffer and homogenized with three 10-s bursts of a Polytron homogenizer (Brinkmann Instruments, Westbury, NY) using a 1-cm probe at a setting of 5. The homogenate was filtered through a double layer of cotton mesh, and the filtrate was centrifuged at 600 g for 5 min. The resulting supernatant was centrifuged at 14,000 g for 10 min, and the supernatant (cytosol-enriched fraction) was decanted and stored at −20°C for later analysis of glutaminase activity. The pellet was washed in 1 ml of buffer (0.1 M KCl and 0.05 M Tris, pH 7.4) and centrifuged again at 14,000 g for 10 min. The supernatant was discarded and the pellet (mitochondrial-enriched fraction) was resuspended in 0.3 ml of buffer, sonicated for 30 s at a setting of 2 with a Heat Systems sonifier (Plainview, NY), and stored frozen at −20°C for analysis of glutaminase activity. Glutamine synthetase and phosphate-dependent glutaminase activities were determined with specific radioisotopic assays similar in principle to those described by Prusiner and Milner (22). Amino acid profiles were also determined on plasma and muscle samples from five of these animals before (day 0) and after (day 8) dexamethasone administration.

Determination of blood flow. Before the determination of hindquarter blood flow and arteriovenous differences, the animals were trained to stand quietly in a Pavlov stand. Food was withheld starting at 5:00 P.M. the day prior to investigation, and all studies were conducted between 8:00 and 11:00 A.M. The animal was placed in a Pavlov stand, and access to the circulation was obtained by percutaneous puncture of the implanted catheter injection ports with 21-gauge needles. A solution of p-aminohippuric acid (PAH; 0.5%, wt/vol, in saline) was infused at a rate of 0.76 ml/min with a Harvard pump into the distal aorta through the catheter in the median sacral artery. After 40 min, simultaneous arterial and venous samples were obtained either by continuous withdrawal over a period of 15 min or as three sets drawn at 10 min intervals. The catheters were then flushed and filled with heparin, and the animal was returned to its kennel.

The concentration of PAH in the arterial and venous samples was determined spectrophotometrically following deproteinization with 5% trichloroacetic acid (14). Blood flow was then calculated as follows

\[
\text{hindquarter blood flow in ml/min} = \frac{(\text{PAH infusion rate in mg/min})}{(\frac{(\text{mg PAH/ml venous blood}) - (\text{mg PAH/ml arterial blood})}{\text{(mg PAH/ml arterial blood)})}}
\]

Hindquarter blood flow was divided by the body weight in kilograms to correct for variation in size of the animals. In studies in which three sets of samples were drawn at 10-min intervals, the blood flow and flux were calculated for each set, and the mean of the three values was determined (12, 14).

Analytical techniques. Whole blood and plasma samples were deproteinized by adding an equal volume of ice-cold perchloric acid (10%, wt/vol) and then centrifuging at 4°C for 20 min. A 2-ml aliquot of the supernatant was buffered with sodium acetate, adjusted to pH 4.75-4.90 with 5 N potassium hydroxide, brought up to 4 ml with water, and stored at −20°C for determination of glutamine and glutamate concentration by a modification of the method from Bergmeyer (19). The remainder of the acid supernatant was stored for amino acid analysis on a Beckman 119 amino acid analyzer using standard ninhydrin methodology. When indicated, substrate fluxes were determined as follows

\[
\text{substrate flux in } \mu\text{mol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} = (\text{arterial concn in } \mu\text{mol/ml}) - (\text{venous concn in } \mu\text{mol/ml}) \times (\text{blood flow in ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1})
\]

At the time of removal of muscle biopsies, a stopwatch was started. The muscle was dissected free of visible fat and connective tissue and divided into two portions, both of which were serially weighed at least four times over a 2-min period. Actual muscle weight at the time of biopsy was calculated from the best-fit linear regression of weight versus time. One sample (approx 15 mg) was dried.
at 90°C, and the weight of dry fat-free solids was determined after extraction in petroleum ether. This sample was then digested in 250 μl of 1 N nitric acid, and the chloride content was measured by titration with silver nitrate using a semiautomated titrator (Radiometer, Copenhagen). Plasma chloride was also determined, and intracellular and extracellular water was calculated by the method of Bergström et al. (7). The other muscle sample (approx 100 mg) was homogenized in 0.5 ml of ice cold perchloric acid (10%, wt/vol) using a Polytron homogenizer. The homogenate was centrifuged, and the supernatant was prepared for amino acid analysis and enzymatic glutamine and glutamate analysis as described above.

A number of control studies were carried out to ensure that glutamine extraction from muscle biopsies was complete and that significant degradation of glutamine did not occur during processing, storage, or analysis of the extracts. It was found that Polytron homogenization of muscle biopsies resulted in complete extraction of glutamine, based on comparison with experiments in which muscle was repeatedly extracted with perchloric acid. Recovery of added glutamine was complete, indicating that there was no significant degradation of glutamine during brief homogenization in 10% perchloric acid at 0–4°C. When extracts are adjusted to pH 4.75–4.90 with KOH and stored at −20°C, glutamine has been shown to be stable for at least 8 wk.

RESULTS

Nitrogen balance. The four dogs studied consumed the same quantity of food during the control and experimental periods, resulting in essentially constant daily nitrogen intake throughout the study (Fig. 1 and Table 1). Urinary nitrogen excretion rose from 0.661 ± 0.038 g N·day⁻¹·kg⁻¹ (mean ± SE) during the control period to 1.009 ± 0.019 g N·day⁻¹·kg⁻¹ during the dexamethasone treatment period (P < 0.01). Stool nitrogen losses increased only slightly with treatment (Table 1).

The animals were in slightly positive nitrogen balance during the control period, +0.126 ± 0.038 g N·day⁻¹·kg⁻¹, but developed markedly negative nitrogen balance during treatment with dexamethasone, averaging −0.278 ± 0.011 g N·day⁻¹·kg⁻¹ (P < 0.01 vs. control period). Cumulative nitrogen loss for these four animals during the 7 days of dexamethasone administration averaged 49.9 g of nitrogen per animal (range 43.7–55.8 g). In spite of the losses of body nitrogen, body weight did not change significantly during the study.

Effect of dexamethasone on plasma and muscle amino acids. To assess the effect of dexamethasone on extracellular and skeletal muscle intracellular free amino acid concentrations, the amino acid profiles in both compartments were measured in five animals prior to treatment (day 0) and following 7 days of dexamethasone therapy (day 8). Two significant alterations occurred in plasma concentrations with treatment (Table 2). Glutamine concentration decreased from 666.0 ± 64.8 to 463.0 ± 44.7 μmol/liter (P < 0.05), and alanine concentration increased from 534.4 ± 111.0 to 797.2 ± 77.0 μmol/liter (P < 0.05). The total concentration of amino acids in plasma was unaltered; the sum of the plasma amino acids was 3.33 ± 0.51 mmol/liter before treatment and 3.61 ± 0.15 mmol/liter following 8 days of dexamethasone therapy. Total plasma amino acid nitrogen also did not change: 4.67 ± 0.68 mmol/liter before treatment compared with 4.85 ± 0.20 mmol/liter following therapy.

In the intracellular compartment of the vastus lateralis muscle, two significant alterations in amino acid concentrations occurred with dexamethasone treatment. Glutamine concentration fell from 17.10 ± 1.13 to 9.13 ± 2.04 mmol/liter intracellular water (P < 0.05). Arginine decreased from 0.67 ± 0.03 to 0.30 ± 0.08 mmol/liter (P < 0.05). Although there was not a statistically significant decline in the intracellular amino acid pool (54.38 ± 4.00 mmol/liter in the controls and 44.41 ± 2.27 mmol/liter

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**Table 1. Effect of dexamethasone on nitrogen balance**

<table>
<thead>
<tr>
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<th>Control</th>
<th>Dexamethasone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen intake, g N·day⁻¹·kg⁻¹</td>
<td>0.994 ± 0.030</td>
<td>0.972 ± 0.002</td>
</tr>
<tr>
<td>Nitrogen output, g N·day⁻¹·kg⁻¹</td>
<td>0.874 ± 0.058</td>
<td>1.250 ± 0.012*</td>
</tr>
<tr>
<td>Urine nitrogen excretion</td>
<td>0.661 ± 0.038</td>
<td>1.009 ± 0.019*</td>
</tr>
<tr>
<td>Stool nitrogen excretion</td>
<td>0.212 ± 0.024</td>
<td>0.248 ± 0.019</td>
</tr>
<tr>
<td>Nitrogen balance, g N·day⁻¹·kg⁻¹</td>
<td>+0.126 ± 0.038</td>
<td>−0.278 ± 0.011*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 4. Control period = 4 days; dexamethasone treatment = 7 days. The mean value for each animal during the control period was compared by paired t test with the mean value during the treatment period. * P < 0.01.

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**FIG. 1.** Effect of glucocorticoids on body weight and nitrogen balance in 4 dogs (mean ± SE). Nitrogen intake is plotted upward from 0 and output downward from top of intake line. Positive balance is indicated by stippled area, while negative balance is shown by solid area.
with dexamethasone treatment), there was a significant fall in intracellular free amino acid nitrogen from 75.02 ± 5.40 to 57.92 ± 3.47 mmol/liter (P < 0.05). This change was primarily the result of altered glutamine concentration, with the decrease in glutamine nitrogen representing 82% of the total change in intracellular amino acid nitrogen.

**Time course of dexamethasone effects on glutamine levels.** The time course of dexamethasone effects on the concentrations of glutamine in venous plasma, whole blood, and skeletal muscle was determined by obtaining samples at intervals during a 14-day treatment period. Since brief general anesthesia was required for each of the sequential muscle biopsies, four control animals were given daily injections of saline and anesthetized and biopsied in the same manner as the dexamethasone-treated animals. Glutamine concentrations, whether measured in plasma or whole blood, showed a progressive decline during 14 days of treatment with dexamethasone (Fig. 2). The intracellular concentration of glutamine also decreased progressively throughout the 14 days. These concentration changes did not appear to plateau within the time frame of the experiment.

**Glutamine synthetase and glutaminase activities.** To gain insight into the mechanism of the marked changes in skeletal muscle intracellular glutamine, the activities of the principal synthetic and degradative enzymes (glutamine synthetase and phosphate-dependent glutaminase) were measured in nine animals before and after 8 days of dexamethasone therapy. Both glutamine synthetase and phosphate-dependent glutaminase activities were present in the vastus lateralis muscle of the dog. Glutamine synthetase activity (apparent $V_{\text{max}} = 93.9 \mu\text{mol} \cdot \text{h}^{-1} \cdot \text{mg cytosol prot}^{-1}$; apparent $K_m = 2.96 \text{mM}$) was limited to the cytosolic compartment, and glutaminase activity (apparent $V_{\text{max}} = 38.5 \mu\text{mol} \cdot \text{h}^{-1} \cdot \text{mg mitochondrial prot}^{-1}$; apparent $K_m = 4.15 \text{mM}$) was contained entirely within the mitochondria. When the apparent $V_{\text{max}}$ of each of the two enzymes was expressed as units per gram wet weight of muscle (1 U = 1 nmol $\cdot$ h$^{-1}$ $\cdot$ g wet wt$^{-1}$), the activity of glutamine synthetase was

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**Table 2. Effect of dexamethasone on plasma and skeletal muscle intracellular amino acid concentrations**

<table>
<thead>
<tr>
<th></th>
<th>Plasma, µmol/liter</th>
<th>Intracellular, mmol/liter</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 8</td>
</tr>
<tr>
<td>Taurine</td>
<td>108.4 ± 14.1</td>
<td>192.5 ± 53.6</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>13.5 ± 1.1</td>
<td>17.5 ± 1.5</td>
</tr>
<tr>
<td>Threonine</td>
<td>273.2 ± 43.4</td>
<td>261.2 ± 37.7</td>
</tr>
<tr>
<td>Serine</td>
<td>130.0 ± 13.5</td>
<td>172.8 ± 41.2</td>
</tr>
<tr>
<td>Asparagine</td>
<td>54.8 ± 10.1</td>
<td>76.4 ± 6.4</td>
</tr>
<tr>
<td>Proline</td>
<td>182.0 ± 23.5</td>
<td>158.0 ± 38.7</td>
</tr>
<tr>
<td>Glycine</td>
<td>208.4 ± 22.0</td>
<td>185.7 ± 11.9</td>
</tr>
<tr>
<td>Alanine</td>
<td>37.0 ± 19.8</td>
<td>34.8 ± 4.3</td>
</tr>
<tr>
<td>Valine</td>
<td>189.6 ± 44.1</td>
<td>233.6 ± 9.2</td>
</tr>
<tr>
<td>Cystine</td>
<td>60.4 ± 6.8</td>
<td>48.0 ± 4.5</td>
</tr>
<tr>
<td>Methionine</td>
<td>62.4 ± 13.0</td>
<td>64.8 ± 5.6</td>
</tr>
<tr>
<td>Glutamate</td>
<td>55.5 ± 16.6</td>
<td>76.0 ± 6.8</td>
</tr>
<tr>
<td>Ornithine</td>
<td>176.0 ± 57.2</td>
<td>240.8 ± 19.8</td>
</tr>
<tr>
<td>Lysine</td>
<td>880.0 ± 13.5</td>
<td>104.0 ± 7.4</td>
</tr>
<tr>
<td>Arginine</td>
<td>139.6 ± 90.3</td>
<td>117.6 ± 15.1</td>
</tr>
<tr>
<td>Glutamine</td>
<td>656.0 ± 64.8</td>
<td>463.0 ± 44.7*</td>
</tr>
<tr>
<td>Phosphoserine</td>
<td>27.6 ± 4.12</td>
<td>16.0 ± 0.8</td>
</tr>
<tr>
<td>Phosphoserine</td>
<td>14.4 ± 2.8</td>
<td>16.0 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5. *Significantly different from day 0, P < 0.05 by paired t test.
found to be more than 40-fold higher than the activity of glutaminase: glutamine synthetase = 3,195.4 nmol·h⁻¹·g wet wt⁻¹ and glutaminase = 76.2 nmol·h⁻¹·g wet wt⁻¹.

After treatment with dexamethasone, no statistically significant alterations in enzyme activities were found (Table 3). The apparent decrease in mean glutaminase activity resulted from a marked fall in glutaminase activity in three of the nine animals; no consistent change in extent or direction of enzyme activity was observed in the additional six animals studied. Addition of glutamine directly to the glutamine synthetase assay did not alter enzyme activity (data not shown), indicating that increased activity of glutamine synthetase in situ could not have occurred simply as a result of declining glutamine concentrations.

**Skeletal muscle amino acid flux.** Because the decrease in intracellular concentration of glutamine in skeletal muscle could not be ascribed to changes in activities of the synthetic or degradative enzymes, hindquarter blood flow and flux determinations were carried out before and during dexamethasone treatment to estimate the rates of efflux of glutamine and other amino acids from skeletal muscle. The average blood flow through the hindquarter did not change following treatment with dexamethasone (Table 4). Mean blood flow was 35 ± 7 ml·min⁻¹·kg⁻¹ during the control period and 26 ± 3 ml·min⁻¹·kg⁻¹ during the dexamethasone period (difference not significant). The mean arteriovenous difference for whole blood glutamine across the hindquarter was -9 ± 7 µmol/liter. This value was not significantly different from zero, indicating that the hindquarter tissues were near glutamine balance during the resting postabsorptive period. After dexamethasone treatment, the arteriovenous difference for glutamine increased significantly to -49 ± 6 µmol/liter, indicating that the hindquarter tissues were not in glutamine balance during the resting postabsorptive period.

<table>
<thead>
<tr>
<th>TABLE 4. Blood flow, arterial concentration, arteriovenous differences, and flux of selected amino acids</th>
<th>Control</th>
<th>Dexamethasone</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. animals</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Avg no. studies/dog (range)</td>
<td>3 (2-4)</td>
<td>2 (2-7)</td>
</tr>
<tr>
<td>Mean wt., kg</td>
<td>26.7 ± 1.9</td>
<td>26.3 ± 1.8</td>
</tr>
<tr>
<td>Hindquarter blood flow, liter·min⁻¹·kg⁻¹</td>
<td>0.035 ± 0.007</td>
<td>0.026 ± 0.003</td>
</tr>
<tr>
<td>Glutamate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial concn, µmol/liter</td>
<td>649 ± 51</td>
<td>671 ± 55</td>
</tr>
<tr>
<td>A-V difference, µmol/liter</td>
<td>-9 ± 7</td>
<td>-49 ± 6*</td>
</tr>
<tr>
<td>Flux, µmol·min⁻¹·kg⁻¹</td>
<td>-0.351 ± 0.165</td>
<td>-1.388 ± 0.304†</td>
</tr>
<tr>
<td>Alanine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial concn, µmol/liter</td>
<td>44 ± 1</td>
<td>51 ± 4†</td>
</tr>
<tr>
<td>A-V difference, µmol/liter</td>
<td>-2 ± 1†</td>
<td></td>
</tr>
<tr>
<td>Flux, µmol·min⁻¹·kg⁻¹</td>
<td>-0.134 ± 0.039</td>
<td>-0.049 ± 0.036*</td>
</tr>
</tbody>
</table>

Values are means ± SE. A-V, arteriovenous. For each dog the control values were averaged, as were the data obtained during dexamethasone administration. Paired t tests were used to compare these seven data sets for each measurement. † Significantly different from control, P < 0.05 by paired t test. * Significantly different from control, P < 0.01 by paired t test.

**DISCUSSION**

The effects of dexamethasone on nitrogen and amino acid metabolism in the dog were studied. This synthetic glucocorticoid with minimal mineralocorticoid activity was chosen because of its ease of administration by intramuscular injection and its prolonged duration of action. A dose of 0.44 mg·kg⁻¹·day⁻¹ of dexamethasone is equivalent to 11 mg·kg⁻¹·day⁻¹ of hydrocortisone, representing approximately a ninefold increase above basal cortisol secretion rate in the dog (31). This dose is approximately five times greater than the 2-2.5 mg·kg⁻¹·day⁻¹ of cortisone recommended for basic maintenance of the adrenalectomized dog (16). A three- to fourfold increase in urinary cortisol can occur following major operation (10), and prolonged elevations in blood cortisol concentrations of at least three to five times control levels have been reported in patients with major burns (29). Thus, the dose of dexamethasone given in this study reasonably approximates the secretion of adrenal steroids during a major stress state.

After dexamethasone administration, the excretion of urinary nitrogen increased in the animals. Since nitrogen intake did not change, steroid therapy thus resulted in a shift from slightly positive to markedly negative nitrogen balance. This finding is consistent with previous studies in which glucocorticoids were administered in the fed state.
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state (9). Dexamethasone also altered plasma amino acid concentrations as reported by others (9, 25). In the present study, plasma glutamine decreased and alanine increased, with total amino acid nitrogen remaining constant.

Intracellular amino acid concentrations in skeletal muscle were also affected by glucocorticoid treatment. This was primarily characterized by a fall in the intracellular concentration of glutamine, although arginine also decreased significantly. Unlike the total plasma amino acid nitrogen, which remained stable, intracellular free amino acid nitrogen was reduced 20–25%, with more than 80% of the nitrogen loss accounted for by the decline in intracellular glutamine. Similar changes in skeletal muscle free amino acid concentrations have been reported following steroid administration in the rat (25).

The decline in intracellular glutamine concentration could result from accelerated release of glutamine from muscle, decreased synthesis of glutamine in muscle, or increased degradation of glutamine in muscle. Therefore, glutamine flux across the hindquarter was determined in resting, unanesthetized dogs. During the control studies, there was a slight efflux of glutamine from muscle that could not be distinguished statistically from zero balance. With dexamethasone treatment, glutamine efflux increased approximately fourfold. The release of glutamine increased within 48 h of glucocorticoid administration and appeared to remain elevated to the same extent during 8 days of treatment in spite of the declining levels of glutamine in muscle. It is likely that the decrease in muscle glutamine concentration following glucocorticoid treatment resulted from the accelerated glutamine efflux.

During dexamethasone treatment, glutamine efflux from the hindquarter averaged 20–25 mmol/day, whereas the entire intracellular glutamine pool in the hindquarter does not exceed 75–100 nmol. Since glutamine comprises only a minor portion of skeletal muscle proteins (17), an increase in net glutamine synthesis must have occurred during dexamethasone treatment. In spite of this increase in net glutamine synthesis, the measured activities of the major synthetase and degradative enzymes in skeletal muscle were unaltered. This was unexpected since both low glutamine concentrations and glucocorticoids have been shown to result in the induction of glutamine synthetase in a number of cultured cell types (28). The measured activities of glutamine synthetase and glutaminase, which were determined in muscle extracts in the presence of optimal concentrations of cofactors, apparently do not reflect actual rates of glutamine synthesis or degradation in vivo.

If it is assumed that the apparent V_max represents the maximum possible in situ activity of glutaminase in dog skeletal muscle (0.57 μmol·min⁻¹·kg body wt⁻¹), it is clear that the observed changes in glutamine efflux could not have resulted from inhibition of glutaminase. Total inhibition of this activity would account for only 50% of the increase in glutamine efflux (Δ = 1.037 μmol·min⁻¹·kg⁻¹) following glucocorticoid treatment. The apparent V_max of glutamine synthetase (23.97 μmol·min⁻¹·kg body wt⁻¹), however, is more than 40-fold higher than glutaminase. If glutamine synthetase were to function at less than 2% of the apparent V_max, this could account for glutamine efflux in the control state (0.351 μmol·min⁻¹·kg⁻¹). An increase to approximately 7% of V_max would account for the accelerated glutamate synthesis in response to dexamethasone. Thus, although additional studies will be required to elucidate the mechanism of increased glutamate synthesis, it is reasonable to hypothesize that it occurs through changes in flux through glutamine synthetase rather than changes in the very low activity of muscle glutaminase.

The ratio of intracellular to extracellular glutamine showed a tendency to decrease during dexamethasone treatment, although the change was not statistically significant. Therefore, the accelerated flux of glutamine from skeletal muscle could not have resulted simply from an increase in the intracellular/extracellular gradient. This implies that glucocorticoids may have altered the amino acid uptake and/or release mechanisms at the plasma membrane. It is interesting to note that alanine efflux also increased in the absence of a change in the gradient across the cell membrane. Therefore, the presumed effect of dexamethasone on amino acid transport involves amino acids that are thought to be handled by different carrier systems (15).

Glutamine efflux from skeletal muscle increased as plasma concentration declined, suggesting that accelerated glutamine uptake occurred at some other site during glucocorticoid therapy. In studies published elsewhere (26), the organs involved in glutamine consumption have been identified, and net amino acid exchange has been quantitated. Studies in dogs in the postabsorptive state reveal that glutamine is taken up by the gastrointestinal tract (0.75 ± 0.17 μmol·min⁻¹·kg⁻¹) and kidney (0.64 ± 0.10 μmol·min⁻¹·kg⁻¹; unpublished data), while there is near balance or slight release from the liver (−1.59 ± 0.59 μmol·min⁻¹·kg⁻¹). After administration of dexamethasone at doses similar to those utilized in this study, the gastrointestinal tract and kidney increase glutamine uptake two- to threefold, while liver remains in balance (or exhibits slight release). In qualitative terms, the rates of glutamine release from skeletal muscle and liver are matched by the accelerated uptake of glutamine by the gastrointestinal tract and kidney.

In addition to glutamine, alanine represents the other major amino acid in the transport of nitrogen from skeletal muscle to other organs of the body (11). In two dogs (data not shown) total amino acid flux across the hindquarter was determined, and glutamine and alanine were found to account for approximately two-thirds of the amino acid nitrogen released. Alanine levels in skeletal muscle did not decline, however, in spite of accelerated efflux during glucocorticoid treatment. Increased alanine release from skeletal muscle probably is related to accelerated hepatic glucoseogenesis associated with hypercorticoidism, which provides increased glucose-derived pyruvate in muscle. Alternatively, some of the alanine carbons as well as nitrogen are thought to be derived from other amino acids that arose from accelerated proteolysis (18).

The majority of glutamate carbons as well as nitrogen are thought to be derived from other amino acids made...
available by accelerated net degradation of muscle protein (11). Thus, glutamine may be more effective than alanine as a vehicle for the unidirectional transfer of both carbon and nitrogen from skeletal muscle to visceral organs. The large intracellular glutamine pool (approximately 20 mmol/liter intracellular water in skeletal muscle) may in fact be a reservoir that is tapped in critical illnesses, perhaps through the influences of elevated glucocorticoid levels.

Glucocorticoid administration probably results in alterations in the concentrations of other hormones (increases in insulin and glucagon) and substrates (glucose and free fatty acids) (9). Thus, the changes observed in this study may be the result of both direct and indirect effects of glucocorticoids. It should be recognized, however, that many of these alterations in response to glucocorticoids are similar to changes observed during critical illness. Stress-related increases in endogenous cortisol secretion are usually associated with hyperglucagonemia, hyperinsulinemia, decreased peripheral tissue sensitivity to insulin, hyperglycemia, increased adrenergic nervous system outflow, and altered fatty acid mobilization (30).

Whether primary or secondary effects, the changes in amino acid levels that are brought about by glucocorticoid treatment parallel, in many respects, alterations that occur in humans with critical illnesses. Specifically, there is a similar fall in intracellular glutamine levels and thus in total free amino acid nitrogen in skeletal muscle following major surgery, trauma, burns, or severe infections (3, 23). This is associated with accelerated amino acid efflux from skeletal muscle and negative nitrogen balance. These findings suggest that the high circulating levels of glucocorticoids in these various disease states may be responsible for the changes in glutamine concentrations and metabolism. In critically ill patients, however, a profound decline in intracellular amino acids, a fall in total plasma amino acids (including alanine), and a rise in intracellular concentrations of specific amino acids, such as the branched-chain amino acids, imply the influence of additional factors. These probably include the multiple hormones that are elevated in response to stress, changes in the metabolism of other substrates, and other factors such as inadequate nutrition, pharmacological agents, and specific effects of the disease itself.

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