Role of Cortisol in the Metabolic Response to Stress Hormone Infusion in the Conscious Dog

Toshihiko Fujiwara, Alan D. Cherrington, Doss N. Neal, and Owen P. McGuinness

The role of cortisol in directing the metabolic response to a combined infusion of glucagon, epinephrine, norepinephrine, and cortisol (stress hormones) was investigated. Chronically catheterized, conscious fasted dogs were studied before hormone infusion and after a 70-hour stress hormone infusion containing glucagon, epinephrine, norepinephrine, and cortisol (n = 11) or containing all these hormones except cortisol (n = 5). Combined stress hormone infusion increased arterial plasma glucagon, cortisol, epinephrine, and norepinephrine approximately sixfold. Whole-body glucose production (R_a), glycogenolysis, and gluconeogenesis were assessed using tracer and arteriovenous-difference techniques. The absence of an increase in cortisol during stress hormone infusion attenuated the increase in arterial plasma glucose concentration and R_a (Δ 81 ± 16 *v* 24 ± 3 mg/dL and 1.7 ± 0.3 *v* 0.8 ± 0.4 mg/kg/min, respectively). However, it did not alter the increase in net hepatic glucose output (Δ 0.7 ± 0.3 *v* 0.8 ± 0.4 mg/kg/min). When the increase in cortisol was absent, the increase in net hepatic gluconeogenic precursor uptake was attenuated (Δ 0.7 ± 0.3 *v* 0.1 ± 0.3 mg glucose/kg/min) due to a decrease in gluconeogenic precursor levels. The efficiency of gluconeogenesis increased to a greater extent (Δ 0.19 ± 0.07 *v* 0.31 ± 0.11) when cortisol was not infused. The absence of an increase in cortisol also led to marked glycogen depletion in the liver (10 ± 4 *v* 55 ± 10 mg/g liver). Cortisol thus plays a pivotal role in the metabolic response to stress hormone infusion by sustaining gluconeogenesis through a stimulatory effect on hepatic gluconeogenic precursor supply and by maintaining hepatic glycogen availability.

INJURY AND INFECTION lead to a profound activation of the neurohypophyseal-pituitary-adrenal axis that can persist for periods exceeding 4 to 5 days.[1] In association with this activation, hepatic glycogenolysis and gluconeogenesis are accelerated. The role of specific hormones in contributing to the acceleration of hepatic glucose production during chronic stress is unclear.

Chronic (5 days) hypercortisolemia increases whole-body glucose production (R_a), hepatic glycogen content, and net hepatic gluconeogenic precursor uptake, whereas acute increases in cortisol have minimal effects.[2-5] Interestingly, hepatic glucose uptake is also increased, and consequently the net release of glucose by the liver is not increased.[5]

How cortisol might interact with an accompanying elevation in other counterregulatory hormones that are also elevated during stress is unclear. In the acute setting, Eigler et al[6] suggested that cortisol synergized with epinephrine and glucagon to enhance R_a, but they did not control for differences in insulin and glucose levels. Lecavalier et al[7] did not observe a synergism between glucagon and cortisol after preventing the cortisol-induced increase in insulin using somatostatin and controlling for changes in glucose. Unfortunately, a maximally effective dose of glucagon was used, which may have prevented detection of a synergistic effect.

The chronic (as opposed to acute) interaction between cortisol and other counterregulatory hormones also has not been clearly defined. Long-term exposure to dexamethasone (3 days at 3 mg/kg/d) in the dog enhances the acute effects of glucagon and epinephrine on R_a.[8] The inclusion of epinephrine and glucagon during a long-term (3-day) infusion of cortisol in obese humans did not augment the cortisol-induced hyperglycemia or increased nitrogen excretion.[9] R_a was not assessed when cortisol was infused alone. In addition, since they did not infuse glucagon and epinephrine in the absence of an increase in cortisol, they were unable to determine if cortisol enhanced the effects of glucagon and epinephrine. In neither of these studies were the specific effects on hepatic glycogenolysis and gluconeogenesis assessed.

We recently reported that a combined infusion of cortisol, epinephrine, norepinephrine, and glucagon (each increased ~sixfold) for 3 days in the chronically catheterized conscious dog increases arterial glucose levels and hepatic glycogenolysis, gluconeogenesis, and glycogen deposition.[10] The role cortisol plays in modulating hepatic glycogenolysis and gluconeogenesis in this setting is unclear. The present investigation was therefore conducted to determine the role cortisol plays in the alterations in hepatic glucose metabolism (gluconeogenesis and glycogenolysis) observed during long-term stress hormone infusion.

MATERIALS AND METHODS

Animal Preparation

Six experiments were performed in 20-hour–fasted conscious mongrel dogs (22 ± 3 kg), and the data were compared with results from 10 previously reported experiments.[10] The dogs were fed a diet consisting of Kal-Kan meat (Vernon, CA) and Purina dog chow (St Louis, MO) once daily. The composition of the diet was 52% carbohydrate, 31% protein, 11% fat, and 6% fiber based on dry weight. The dogs were housed in a facility that met the guidelines of the American Association for the Accreditation of Laboratory Animal Care, and the protocols were approved by the Vanderbilt University Medical Center animal care committee.

Two weeks before the first experiment, a laparotomy was

From the Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN.

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Address reprint requests to Owen P. McGuinness, PhD, 702 Light Hall, Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN 37232-0615.

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performed under general anesthesia (acepromazine 0.55 mg/kg plus sodium pentobarbital 25 mg/kg). Silastic catheters (0.03-inch ID; Dow Corning, Midland, MI) were placed into the inferior vena cava for long-term infusion of hydrocortisone, epinephrine, and norepinephrine and into a splenic vein for long-term infusion of glucagon. Blood-sampling catheters (0.04-inch ID) were inserted into the femoral artery, portal vein, right renal vein, and left hepatic vein. In 12 dogs, Doppler flow probes were placed about the portal vein and hepatic artery after the gastroduodenal vein was ligated.[10] In four dogs, Doppler flow probes were not used. The sampling and infusion catheters and Doppler leads were placed under the skin, and the wounds were closed. The dogs received penicillin G intramuscularly (10^6 U) immediately after wound closure to minimize the possibility of infection. All animals studied had (1) a good appetite (consuming all of the daily ration), (2) normal stools, (3) a hematocrit level greater than 35%, and (4) a leukocyte count less than 18,000/μL on both experiment days.

Experimental Design

Basal metabolism was assessed on day 0 after a 20-hour fast. At the end of the experiment, the dog was placed in a jacket (Alice King Chatham, Los Angeles, CA) containing two pockets, each of which held a portable infusion pump (Auto Syringe; Travenol Laboratories, Hooksett, NH). Hydrocortisone was dissolved in saline and infused in 11 dogs at a rate of 4 μg/kg/min (240 μL/h) into the inferior vena cava (SHI + CORT), and in five other dogs, saline was infused in place of the hydrocortisone for 3 days (SHI − CORT). In all 16 dogs, epinephrine and norepinephrine were dissolved in saline containing ascorbic acid (0.7 mg/mL) and both were infused at a rate of 0.08 μg/kg/min (240 μL/h) into the inferior vena cava. Glucagon (5 ng/kg/min) was infused into the portal vein using a swivel-tether system (Alice King Chatham). All solutions were prepared and filtered (0.2 μm) under sterile conditions before infusion, as previously described.[10] Fresh solutions were prepared every 12 hours on each of 3 infusion days. On the third day following an overnight (20-hour) fast, basal metabolism was reassessed.

Experimental Protocol

On days 0 and 3, an Angiocath (18-gauge; Deseret Medical, Sandy, UT) was inserted percutaneously into a cephalic vein. A primed (50 μCi)-constant infusion of purified [3-^{3}H]glucose (0.4 μCi/min) and constant infusions of [U-^{14}C]alanine (0.4 μCi/min), *p*-aminohippuric acid (0.3 mg/kg/min), and indocyanine green (0.1 mg/m^2/min) were begun using the right cephalic vein and continued throughout the entire experiment. The experiment consisted of two periods, an equilibration period (−120 to 0 minutes) and a basal period (0 to 60 minutes). Femoral artery and portal, renal, and hepatic vein blood samples were taken every 15 minutes during the basal period. At the end of the basal period in the saline protocol (SHI − CORT), the dog was killed and liver biopsies were rapidly (~4 minutes) taken from each lobe of the liver and immediately frozen in liquid nitrogen for later analysis of liver glycogen content and tracer incorporation into glycogen. In the cortisol-infused group (SHI + CORT), the basal period was extended by an additional 180-minute experimental period, after which glycogen samples were obtained as described for the cortisol-deficient group. The glycogen data reported for this group are from five animals. In six of the dogs, glucagon was acutely removed during the experimental period, and the glycogen data could not be used.[11]

Tracer Methods and Calculations

The rates of total glucose production (R_a) and utilization (R_d) were calculated according to the method of Wall et al[12] as simplified by DeBodo et al.[13] Net hepatic glucose output was calculated using the formula, $[H - (F_a \times A + F_p \times P)] \times HBF$, where H, A, and P are blood glucose concentrations in the hepatic vein, femoral artery, and portal vein, respectively, and F_a and F_p represent the fractional contribution of the hepatic artery and portal vein, respectively, to total hepatic blood flow (HBF). Plasma glucose concentrations were converted to whole-blood concentrations using a correction factor of 0.73.[14] The hematocrit was not different between the two groups on day 3 (39% ± 1% *v* 38% ± 1%, SHI + CORT *v* SHI − CORT).

The above equation was used to calculate net hepatic lactate, alanine, and glycerol output. However, since the liver generally is a net consumer of these substrates (ie, negative output), the data are presented as positive values and denote net uptake.

In the present study hepatic blood flow was assessed using Doppler flow probes in six of 11 dogs that received the complete stress hormone infusion and in four of five cortisol-deficient dogs. The fractional contribution of hepatic arterial blood flow to total hepatic blood flow was not altered by stress hormone infusion in either group (0.18 ± 0.04 to 0.17 ± 0.03 in SHI + CORT and 0.20 ± 0.02 to 0.23 ± 0.03 in SHI − CORT from day 0 to day 3). Doppler flow probes were not available for implantation in four dogs in the SHI + CORT group. Doppler flow probes did not function correctly in two dogs (one in each group). In dogs in which we did not have Doppler flow data, the fractional contribution of hepatic artery blood flow was assumed to equal the mean determined with Doppler flow probes in the respective groups, and indocyanine green–determined flows were used.

Net renal glucose output was calculated using the formula, $[R - A] \times RBF$, where R and A are renal vein and femoral artery blood glucose concentrations and RBF is renal blood flow. Net renal lactate, alanine, and glycerol output were calculated using the same formula. Again, since the kidney tended to be a net consumer (ie, negative output) of these precursors, the data are presented as positive values and denote net uptake. Net intestinal lactate, alanine, and glycerol output was calculated using the formula, $[P - A] \times PBF$, where A and P are femoral artery and portal vein blood substrate concentrations and PBF is portal blood flow. To be consistent with net renal and hepatic uptake of these substrates, data for the intestine are also presented as net uptake.

Organ (hepatic, renal, or intestinal) glucose uptake was calculated as the product of organ blood flow and the ratio of the organ net [3-^{3}H]glucose balance to the average inflowing glucose specific activity. This assumes that the specific activity of glucose at the site of glucose utilization is equal to the inflowing glucose specific activity. However, even if this assumption is incorrect, the error is small, since inflowing and outflowing glucose specific activities were within 10% of one another. Although the decrease in specific activity is small, the impact on the calculated rates of organ glucose production and uptake is significant.[10] Organ glucose production is equal to the sum of net organ glucose output and organ glucose uptake.

Hepatic gluconeogenic efficiency was calculated as the ratio of the ^{14}C-glucose production rate to the total net hepatic ^{14}C gluconeogenic precursor uptake rate (alanine and lactate), as described previously.[15] On day 0, the liver (11 of 16 cases) was a net producer of ^{14}C-lactate. However, on day 3, livers of all 16 dogs were ^{14}C-lactate consumers. When the liver was a net ^{14}C-lactate producer, the contribution of ^{14}C-lactate to ^{14}C gluconeogenic precursor uptake was set equal to zero. Inclusion of ^{14}C-lactate in the calculation of gluconeogenic efficiency has little impact on the

results, since the amount of radioactivity in lactate relative to that in alanine was small.

The methods used herein allowed us to bracket the true rate at which circulating gluconeogenic precursors are converted to glucose, as described previously.[10] A maximal estimate of gluconeogenesis was obtained by assuming that all gluconeogenic precursors taken up by the liver were completely converted to glucose. In cases in which the liver was a net lactate producer, net hepatic lactate uptake was not included in the calculation of net hepatic gluconeogenic precursor uptake. A minimal estimate was obtained by multiplying this maximal estimate by the tracer-determined gluconeogenic efficiency (which is itself a minimal estimate, as discussed elsewhere[15,16]).

Processing of Blood Samples

Radioactivity in plasma glucose was measured using established methods.[17] Blood lactate, glycerol, and alanine were analyzed using the method of Lloyd et al.[18] Plasma glucose was assayed immediately using a Beckman glucose analyzer (Fullerton, CA). Individual gluconeogenic amino acids were assessed with reverse-phase high-performance liquid chromatography (HPLC) using a modified version of previously reported methods.[19,20] Plasma treated with 500 kIU Trasylol (FBA Pharmaceuticals, New York, NY) was assayed for immunoreactive glucagon using the 30K antiserum of Unger[21] (coefficient of variation [CV], 8%). Immunoreactive insulin[22] was assayed using a Sephadex-bound antibody technique (Pharmacia Diagnostics, Piscataway, NJ; CV, 11%). Plasma cortisol was assayed with a Clinical Assays (Stillwater, MN) Gamma Coat radioimmunoassay kit[23] (CV, 6%). Plasma collected from blood samples that were immediately treated with EGTA and glutathione was assayed for epinephrine and norepinephrine using HPLC techniques (CV, 14%). Indocyanine green dye was assayed spectrophotometrically (810 nm) to estimate total hepatic blood flow.[24] Doppler-determined blood flow was obtained using an ultrasonic range-gated, pulsed Doppler flow meter designed by Hartley et al.[25] *p*-Aminohippuric acid level was measured as described by Brun[26] to estimate renal blood flow. Labeled and unlabeled alanine and lactate plasma concentrations were determined using a short-column ion-exchange chromatographic system.[15] Hepatic glycogen content was determined using an enzymatic method.[27]

Materials

Glucagon was purchased from Eli Lilly and Co (Indianapolis, IN). Epinephrine, norepinephrine, and *p*-aminohippuric acid were obtained from Sigma Chemical (St Louis, MO). Hydrocortisone was purchased from Abbott Laboratories (North Chicago, IL). Glucagon 30K antiserum was obtained from the University of Texas Southwestern Medical School (Dallas, TX). Purified glucagon and ^{125}I-glucagon for radioimmunoassay were obtained from Novo Research Institute (Copenhagen, Denmark). Cortisol assay kits were obtained from Upjohn Diagnostics (Kalamazoo, MI). [3-^{3}H]glucose (HPLC-purified) and [U-^{14}C]alanine were purchased from New England Nuclear Research Products (Wilmington, DE).

Data Analysis

The data represent the mean ± SEM of steady-state values during the basal period on days 0 and 3. Data for the SHI + CORT group have been previously reported,[10] and are presented to facilitate comparisons between the two groups. Four of 11 studies in the SHI + CORT group were performed simultaneously in the SHI − CORT group. Since the response to stress hormone infusion (SHI + CORT) in the four studies was similar to that previously reported, the data were combined. Statistical comparisons were made with two-way ANOVA using a statistical software package (SYSTAT for Windows; Systat, Evanston, IL).

RESULTS

All the data from the SHI + CORT group have been previously reported[10] and are presented to facilitate comparisons between the two groups.

Hormone Levels and Glucose Metabolism

Stress hormone infusion (SHI + CORT) increased arterial plasma glucagon, epinephrine, norepinephrine, and cortisol approximately sixfold (Table 1). When cortisol was not infused (SHI − CORT), plasma cortisol level was not altered but arterial plasma catecholamine and glucagon levels increased approximately sixfold. Arterial plasma insulin increased markedly (26 ± 5 μU/mL, $P < .05$) on day 3 in SHI + CORT (Table 1), whereas it increased only slightly (5 ± 2 μU/mL, $P < .05$) in SHI − CORT. In SHI + CORT, arterial plasma glucose concentration increased by 82 ± 16 mg/dL ($P < .05$, Fig 1); in SHI − CORT, it increased by only 24 ± 3 mg/dL ($P < .05$). R_a increased by 1.7 ± 0.3 mg/kg/min in SHI + CORT and by only 0.8 ± 0.4 mg/kg/min in SHI − CORT. In contrast, net hepatic glucose output and hepatic glucose production increased similarly in SHI + CORT (Δ 0.7 ± 0.2 and 0.6 ± 0.2 mg/kg/min, $P < .05$) and SHI − CORT (Δ 0.8 ± 0.4 and 0.6 ± 0.3 mg/kg/min). R_d increased to a greater extent when cortisol was elevated as opposed to when it was not (Δ 1.9 ± 0.2 *v* 0.6 ± 0.4 mg/kg/min, SHI + CORT *v* SHI − CORT; Fig 1). Glucose clearance was not altered in SHI + CORT or SHI − CORT.

Hepatic Gluconeogenesis

SHI + CORT did not alter arterial blood lactate concentration, but SHI − CORT decreased it markedly ($P < .05$). Net hepatic fractional lactate extraction ($P < .05$) and uptake ($P < .05$) increased in both protocols. However, the increase in net hepatic lactate uptake was attenuated by approximately 50% in SHI − CORT (Δ 4.9 *v* 9.9 μmol/kg/min; Fig 2).

In both protocols, net hepatic fractional alanine extraction increased and arterial blood alanine concentration decreased ($P < .05$). These effects offset one another in

Table 1. Effect of a 3-Day Stress Hormone Infusion With or Without Cortisol on Arterial Plasma Glucagon, Epinephrine, Norepinephrine, Cortisol, and Insulin in 20-Hour–Fasted Conscious Dogs

	SHI + CORT		SHI − CORT	
Variable	Day 0	Day 3	Day 0	Day 3
Glucagon (pg/mL)	46 ± 5	227 ± 13*	56 ± 10	204 ± 14*
Epinephrine (pg/mL)	194 ± 25	1,247 ± 108*	167 ± 79	1,378 ± 124*
Norepinephrine (pg/ml)	215 ± 34	1,271 ± 111*	243 ± 61	1,182 ± 182*
Cortisol (μg/dL)	3 ± 1	13 ± 1*	2 ± 1	3 ± 1
Insulin (μU/mL)	11 ± 1	37 ± 3*	17 ± 2	22 ± 3

NOTE. Data are expressed as the mean ± SE.
*Significantly different from day 0 ($P < .05$).

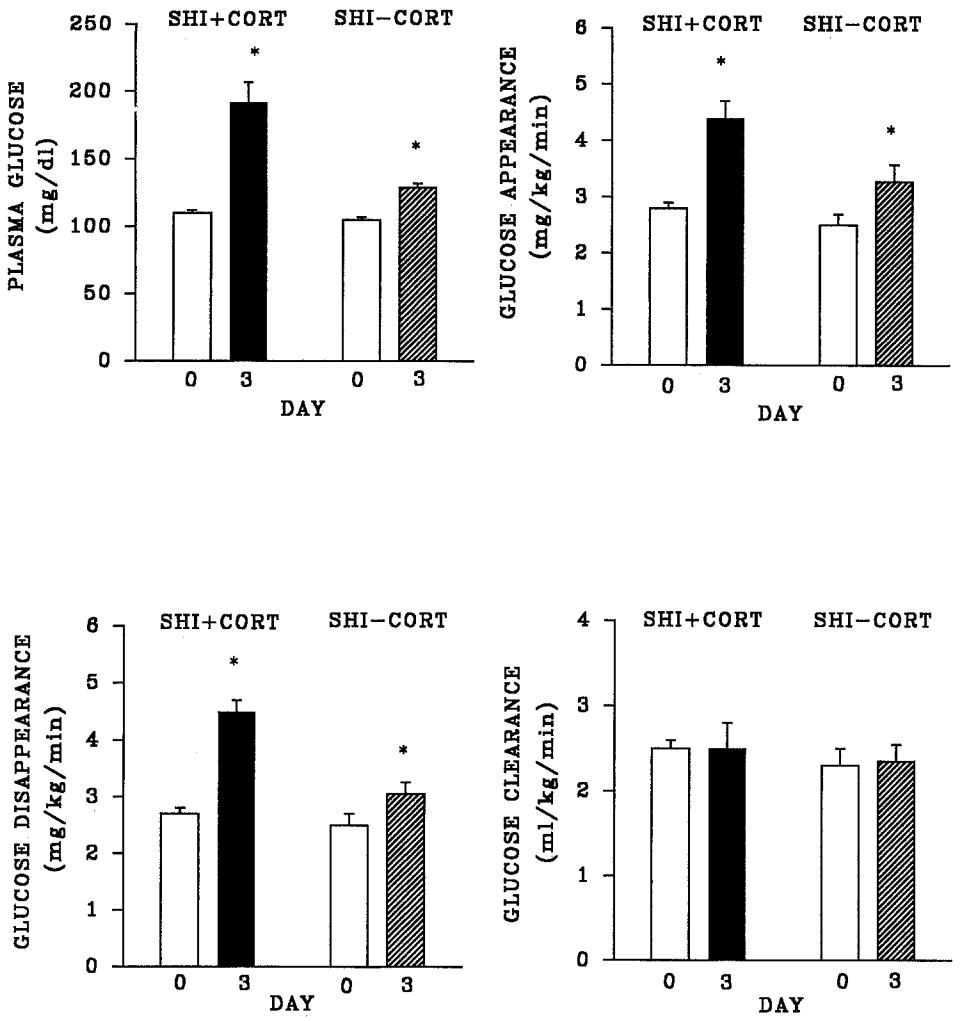

Fig 1. Effect of long-term stress hormone infusion with (SHI + CORT) or without (SHI − CORT) cortisol on arterial plasma glucose concentration and tracer-determined glucose appearance, disappearance, and clearance in 20-hour–fasted conscious dogs. Data are expressed as the mean ± SE. *Significantly different from day 0 ($P < .05$).

SHI + CORT, and consequently net hepatic alanine uptake was not altered. However, in SHI − CORT, arterial plasma alanine concentration decreased to a greater extent, such that net hepatic alanine uptake decreased ($P < .05$; Fig 3).

The combined arterial blood levels of the other gluconeogenic amino acids (glycine, serine, and threonine) decreased markedly in both groups on day 3 (503 ± 21 to 212 ± 15 and 485 ± 27 to 208 ± 12 μmol/L in SHI + CORT and SHI − CORT, respectively). The combined net hepatic uptake of these amino acids increased similarly in both groups (1.6 ± 0.2 to 2.8 ± 0.2 and 1.3 ± 0.3 to 2.2 ± 0.4 μmol/kg/min in SHI + CORT and SHI − CORT, respectively). Arterial glutamine levels also decreased equivalently in both studies. Since the liver remained a net producer of glutamine on days 0 and 3 in both groups, glutamine was not a net contributor to gluconeogenesis (data not presented).

The arterial blood glycerol level and net hepatic glycerol uptake increased slightly in both SHI + CORT and SHI − CORT, but the changes were statistically significant only in SHI + CORT. Fractional extraction of glycerol by the liver was not significantly altered in either group (Fig 4).

SHI + CORT increased total hepatic gluconeogenic precursor uptake (Δ 0.8 ± 0.3 mg glucose equivalents/kg/min, $P < .05$) and gluconeogenic efficiency (Δ 0.25 ± 0.07, $P < .05$). SHI-CORT increased gluconeogenic efficiency (Δ 0.31 ± 0.11, $P < .05$), whereas total hepatic gluconeogenic precursor uptake did not increase. Arterial plasma alanine specific activity was increased following SHI + CORT, and was increased further after SHI − CORT. Arterial lactate specific activity was not altered by SHI + CORT or SHI − CORT (Fig 5 and Table 2).

Hepatic Glycogen

Hepatic glycogen content was markedly decreased in SHI − CORT versus SHI + CORT (10 ± 4 *v* 55 ± 10 mg/g, $P < .05$) and in the previously reported[10] saline-infused control group (30 ± 4 mg/g, $P < .05$).

Renal Substrate Balance

Renal blood flow increased in both SHI + CORT and SHI − CORT (21 ± 1 to 37 ± 5 and 20 ± 3 to 29 ± 4 mL/kg/min, $P < .05$). The kidney was a net consumer of glucose on day 0 in both groups, and uptake increased in response to SHI + CORT (0.2 ± 0.2 to 0.9 ± 0.3 and 0.2 ± 0.1 to 1.2 ± 0.3 mg/kg/min). On day 0, renal glucose production was insignificant in both groups; however,

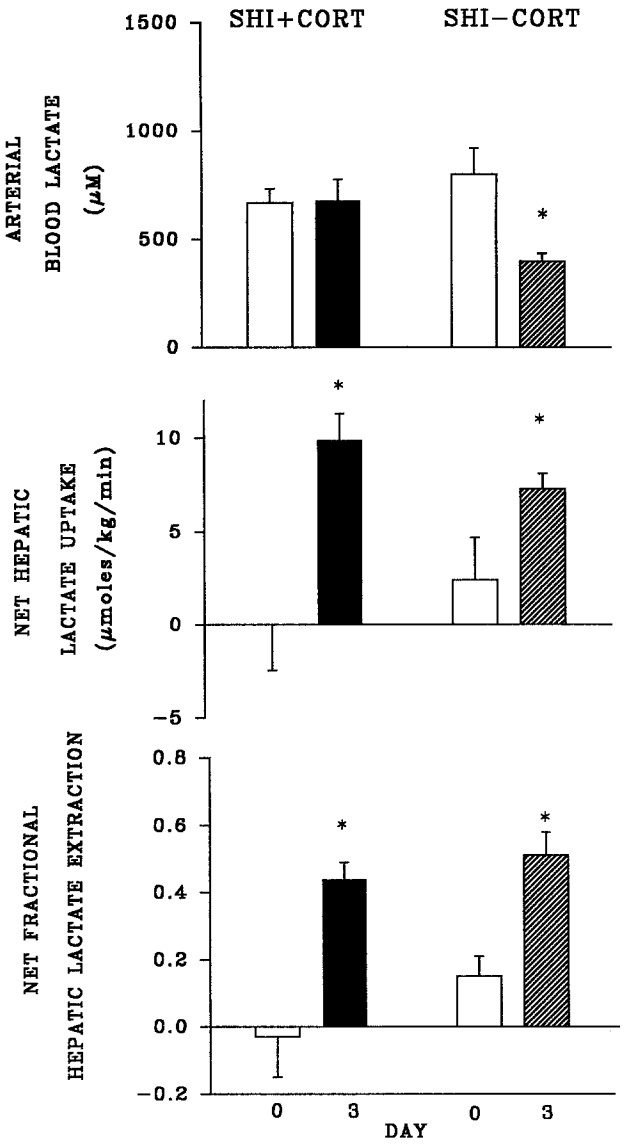

Fig 2. Effect of long-term stress hormone infusion with (SHI + CORT) or without (SHI − CORT) cortisol on arterial blood lactate concentration, net fractional hepatic lactate extraction and net hepatic lactate uptake in 20-hour–fasted conscious dogs. Data are expressed as the mean ± SE. *Significantly different from day 0 ($P < .05$).

following SHI + CORT, renal glucose production increased (0.3 ± 0.3 to 0.9 ± 0.4 mg/kg/min). In SHI − CORT, an increase in renal glucose production did not occur (0.3 ± 0.1 to 0.0 ± 0.1 mg/kg/min).

The kidney was a net consumer of lactate in both groups. Net renal lactate uptake increased in SHI + CORT (3.2 ± 0.5 to 6.3 ± 1.8 μmol/kg/min), but was decreased in SHI − CORT (2.8 ± 0.7 to 1.9 ± 0.8 μmol/kg/min). Net fractional lactate extraction by the kidney was not altered in SHI + CORT (0.23 ± 0.03 to 0.24 ± 0.02), but tended to decrease in SHI − CORT (0.19 ± 0.03 to 0.14 ± 0.07).

The kidney was also a net consumer of glycerol. Net renal glycerol uptake increased in both protocols (0.4 ± 0.1 to 1.0 ± 0.1 and 0.2 ± 0.2 to 0.8 ± 0.3 μmol/kg/min, SHI + CORT and SHI − CORT, $P < .05$). Net renal glycerol fractional extraction was not altered by SHI + CORT (0.15 ± 0.07 to 0.22 ± 0.02) or by SHI − CORT (0.09 ± 0.08 to 0.18 ± 0.06).

The kidney was not a net consumer of alanine, and in fact it produced a small amount of alanine (data not presented). However, the rate of alanine release was too low to assess the effects of stress hormone infusion.

Intestinal Substrate Balance

The intestine was a net consumer of glucose on day 0 in both groups. Net intestinal glucose uptake was not altered on day 3 in SHI + CORT (0.4 ± 0.1 to 0.4 ± 0.2 mg/kg/

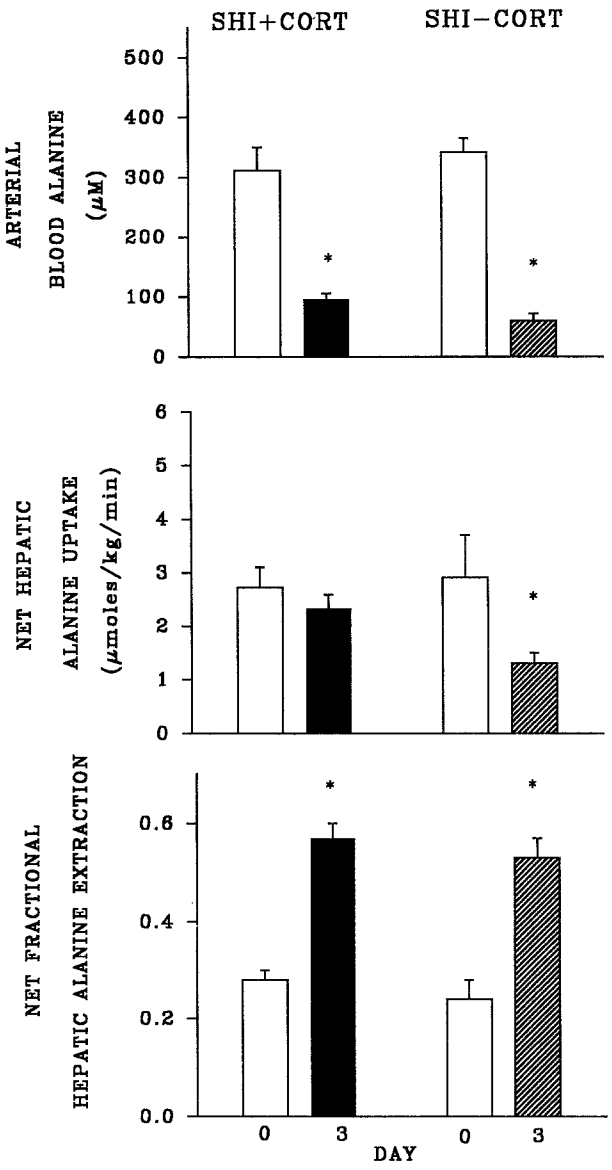

Fig 3. Effect of long-term stress hormone infusion with (SHI + CORT) or without (SHI − CORT) cortisol on arterial blood alanine concentration, net fractional hepatic alanine extraction, and net hepatic alanine uptake in 20-hour–fasted conscious dogs. Data are expressed as the mean ± SE. *Significantly different from day 0 ($P < .05$).

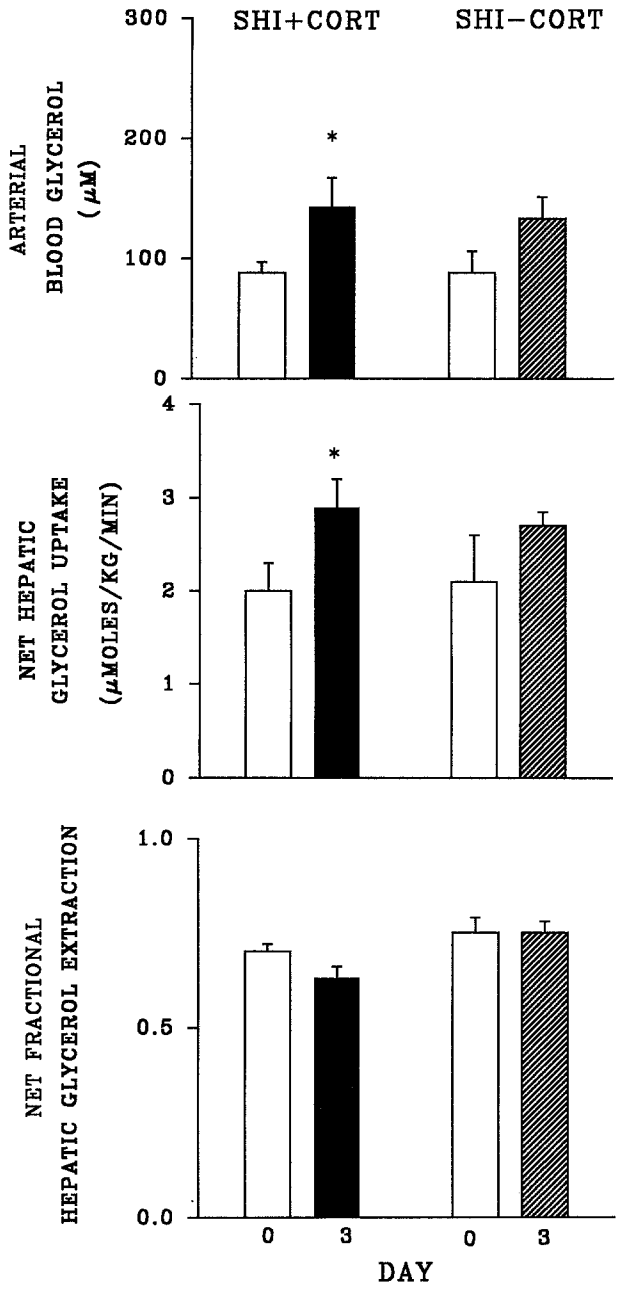

Fig 4. Effect of long-term stress hormone infusion with (SHI + CORT) or without (SHI − CORT) cortisol on arterial blood glycerol concentration, net fractional hepatic glycerol extraction, and net hepatic glycerol uptake in 20-hour–fasted conscious dogs. Data are expressed as the mean ± SE. *Significantly different from day 0 ($P < .05$).

min), but tended to increase in SHI − CORT (0.3 ± 0.2 to 0.5 ± 0.3 mg/kg/min). There was no apparent glucose production by the intestine on day 0 in either group. However, SHI + CORT tended (0.2 ± 0.2 to 0.9 ± 0.3 mg/kg/min, $P = .07$) to increase intestinal glucose production on day 3, whereas SHI − CORT did not (0.1 ± 0.3 to 0.1 ± 0.4 mg/kg/min).

The intestine was a net producer of lactate and alanine in

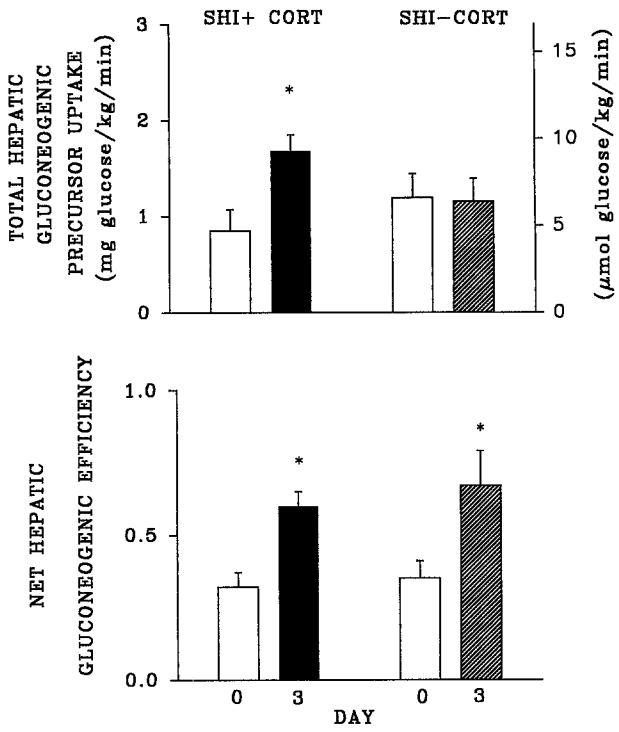

Fig 5. Effect of long-term stress hormone infusion with (SHI + CORT) or without (SHI − CORT) cortisol on net hepatic gluconeogenic precursor uptake and net hepatic gluconeogenic efficiency in 20-hour–fasted conscious dogs. Data are expressed as the mean ± SE. *Significantly different from day 0 ($P < .05$).

both groups. Alanine output by the intestine was not altered by stress hormone infusion (0.9 ± 0.1 to 0.8 ± 0.2 and 1.1 ± 0.2 to 0.7 ± 0.1 μmol/kg/min, SHI + CORT and SHI − CORT). However, intestinal lactate output was increased on day 3 during SHI + CORT (1.0 ± 0.3 to 2.4 ± 0.5 μmol/kg/min, $P < .05$) and SHI − CORT (1.8 ± 0.5 to 2.7 ± 0.4 μmol/kg/min, $P < .05$). Net intestinal glycerol output was not detectable (data not presented).

DISCUSSION

This study demonstrates that cortisol plays an important role in the stimulation of hepatic glucose metabolism seen

Table 2. Effect of a 3-Day Stress Hormone Infusion With or Without Cortisol Upon Alanine and Lactate Specific Activity in the Artery, Portal Vein, and Hepatic Vein in 20-Hour–Fasted Conscious Dogs

	SHI + CORT (n = 11)		SHI − CORT (n = 5)	
Parameter	Day 0	Day 3	Day 0	Day 3
Alanine specific activity				
Artery	6.6 ± 1.1	12.2 ± 1.6*	4.7 ± 0.6	21.9 ± 6.3*
Portal vein	4.6 ± 0.7	5.7 ± 0.6	3.4 ± 0.4	9.4 ± 2.9
Hepatic vein	4.5 ± 0.8	11.5 ± 3.0	3.4 ± 0.6	16.6 ± 5.2
Lactate specific activity				
Artery	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
Portal vein	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
Hepatic vein	0.8 ± 0.2	0.7 ± 0.1	0.5 ± 0.1	0.5 ± 0.1

NOTE. Data are expressed as the mean ± SE (dpm × 10^3/μmol).
*Significantly different from day 0 ($P < .05$).

during long-term stress hormone infusion. The absence of hypercortisolemia during stress hormone infusion led to marked glycogen depletion. Although not altering the increase in net hepatic glucose output seen with stress hormone infusion, the absence of hypercortisolemia prevented the characteristic increase in gluconeogenesis. Thus, the increase in net hepatic glucose output was derived from the accelerated breakdown of the already limited glycogen reserves. The lack of an increase in hepatic gluconeogenesis when hypercortisolemia was not present, despite hyperglucagonemia and elevated catecholamines, was due to a lack of an increase in gluconeogenic precursor supply rather than to an impaired stimulation of the gluconeogenic pathway per se.

Cortisol's ability to enhance glycogen deposition is not prevented by long-term exposure to glycogen-mobilizing hormones. It is known from many classic studies[2] that cortisol replacement in adrenalectomized animals and cortisol infusion in normal animals can markedly augment hepatic glycogen synthesis.[3,5] In the present study, the combined infusion of epinephrine, norepinephrine, and glucagon (SHI − CORT) dramatically decreased hepatic glycogen content. Interestingly, not only did inclusion of cortisol (SHI + CORT) increase the glycogen mass above normal,[10] but the difference in glycogen content between SHI + CORT and SHI − CORT (Δ 45 mg/g liver) was nearly identical to the increase observed when cortisol is infused alone (Δ 46 mg/g liver[5]). Thus, despite markedly elevated catecholamines and glucagon, cortisol maintains the liver's ability to synthesize glycogen, possibly by increasing glycogen synthase activity[2,27,28] and/or by increasing glycogen synthesis via the indirect pathway.[2,28]

An increase in cortisol is required to supply the necessary gluconeogenic precursors to support the stress hormone–induced increase in net hepatic gluconeogenic precursor uptake. In SHI + CORT, gluconeogenic precursor uptake was 0.5 mg glucose/kg/min higher than for SHI − CORT on day 3. Lactate levels decreased (40%) and alanine levels decreased further (35%) in SHI − CORT, presumably due to a decrease in their release by peripheral tissues. Arterial alanine specific activity was higher when cortisol was not infused, which is consistant with a decrease in alanine turnover (Table 2). Although it is known that hypercortisolemia does increase muscle proteolysis,[3,5,9] a decrease in protein breakdown in SHI − CORT is unlikely, since net hepatic uptake of the other gluconeogenic amino acids was not decreased. The most likely explanation for the decrease in alanine availability, as well as lactate availability, is a decrease in muscle glucose uptake. The amount of glucose released by the liver that was not taken up by the kidney and intestine was 43% lower (0.8 *v* 1.4 mg/kg/min) on day 3 when cortisol was not increased as compared with SHI + CORT. Since the predominant fate of this glucose is neural and muscle tissue and it is unlikely that glucose uptake by neural tissues decreased, muscle glucose uptake must have decreased. This decrease was likely due to the lower glucose levels in SHI − CORT.

Combined infusion of glucagon and the catecholamines (SHI − CORT) did not increase gluconeogenesis derived from lactate—instead, it decreased hepatic glycolysis. Generally, overnight-fasted dogs are producers of lactate, as was commonly seen in our experiments on day 0. In animals who were net lactate producers, the contribution of lactate to gluconeogenic precursor uptake was set equal to zero. As a consequence, although net hepatic lactate uptake did increase in SHI − CORT relative to day 0, this increase was due to a decrease in net hepatic lactate release rather than to an augmentation of lactate uptake. Thus, animals that were already hepatic lactate consumers on day 0 did not increase net hepatic lactate uptake further. Animals that were lactate producers on day 0 decreased their release of lactate, but did not switch to marked lactate consumption.

The lack of an increase in cortisol did not affect intrahepatic handling of gluconeogenic precursors. The stress hormone–induced increase in net hepatic fractional extraction of lactate and alanine and in the efficiency of gluconeogenesis by the liver were not attenuated. Previous study demonstrated that the gluconeogenic pathway between phosphoenolpyruvate and pyruvate was enhanced in livers isolated from adrenalectomized animals replaced with glucocorticoids.[28] In vivo chronic hypercortisolemia alone does not enhance the fractional extraction of lactate and alanine by the liver, nor does it increase the efficiency of gluconeogenesis.[5] Since hypercortisolemia is generally associated with increased insulin levels, it is possible that a compensatory increase in insulin limited the cortisol-induced increase in gluconeogenic efficiency.

Surprisingly, the attenuation of R_a when cortisol was not infused was due to a decrease in renal and intestinal glucose production rather than to a decrease in hepatic glucose production. The increases in hepatic glucose production and net hepatic glucose output were not attenuated. Of particular note, neither the kidney nor the intestine was a net producer of glucose; the liver is the sole net source of glucose. How much of this extrahepatic glucose production represents "true" production, as opposed to tracer exchange, is unclear.[30] Cortisol is able to induce the synthesis of rate-limiting gluconeogenic enzymes in the renal cortex.[31] The apparent production of glucose from the intestine is likely due to delayed absorption of the previous meal. The mechanism for this is unclear. However, irrespective of whether the extrahepatic production represents true production, changes in whole-body R_a during stress hormone infusion do not always reflect changes in the release of glucose by the liver.

The liver preferentially removes amino acids delivered from the portal vein rather than from the hepatic artery. Following stress hormone infusion, the hepatic vein alanine specific activity was greater than the weighted inflowing alanine specific activity. A differential increase in hepatic vein alanine specific activity could occur if the liver only removed the alanine with a lower specific activity in the portal vein, leaving behind alanine with a higher specific activity from the hepatic artery. This is most apparent when the fractional extraction of alanine is elevated. A preferen-

tial removal of another amino acid (leucine) delivered via the portal vein for hepatic protein synthesis has been reported.[32]

In summary, cortisol plays a central role during stress hormone infusion by sustaining substrate availability to support an increase in gluconeogenesis. In addition, it preserves hepatic glycogen stores in the face of marked elevations in other counterregulatory hormones.

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