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Early Hormonal Changes Affect the Catabolic Response to Trauma

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Objective
The authors sought to determine how temporary insulin suppression might alter the catabolic effects of cortisol, glucagon, and epinephrine.

Summary Background Data
The metabolic responses to injury include hypermetabolism, accelerated net skeletal muscle protein breakdown, glucose intolerance, and insulin resistance. These alterations are associated with increased stress hormone concentrations. Insulin elaboration is usually suppressed immediately after an injury but is abundant later during convalescence. An infusion of hydrocortisone, glucagon, and epinephrine increases both stress hormone concentrations and insulin levels. It induces many of the metabolic alterations seen in critically ill patients, but it does not affect net muscle breakdown.

Methods
Seven healthy adults received a stress hormone infusion for 3 days in two separate studies. During one study they, also received an infusion of the somatostatin analogue, octreotide (0.005 μg/kg/min), to suppress insulin elaboration for the first 24 hours. During the other study (control), insulin was permitted to rise unchecked.

Results
Stress hormone concentrations, hypermetabolism (± 20% above basal), and leukocytosis were similar during both study periods. When insulin elaboration was temporarily suppressed, whole-body nitrogen loss was increased during the first 48 hours, and the efflux of amino acids from the forearm after 72 hours of infusion was 60% greater than the control level.

Conclusions
Temporary insulin suppression during physiologic increases in stress hormone concentrations amplified whole-body nitrogen loss and led to the development of accelerated net skeletal muscle protein breakdown. Early hormonal changes after an injury may affect the development of later catabolic responses.

The metabolic responses to injury and critical illness include hypermetabolism, accelerated skeletal muscle protein breakdown, glucose intolerance, and insulin resistance. The enhanced proteolysis is especially morbid because it results in marked muscle wasting and debility. If prolonged or intense, protein breakdown and nitrogen loss may be associated with organ failure and death. The mechanisms underlying these responses, especially the accelerated net skeletal muscle protein breakdown, are incompletely defined. A better understanding of them

could lead to improved strategies of care for injured and critically ill surgically treated patients.

For many years, investigators have considered the role of endocrine hormones in regulating the catabolic responses to critical illness.[1] Concentrations of the counterregulatory hormones, cortisol,[2,3] glucagon,[4] and epinephrine[5,6] are usually elevated. Several studies have attempted to relate different aspects of the injury response to the actions of individual hormones,[1,7-10] but the net effect of these hormones when secreted at the same time in patients may not be straightforward. Some of the hormones have opposing effects. For example, cortisol increases net muscle breakdown,[11] but epinephrine promotes amino acid uptake by muscle.[12] One group demonstrated in humans that when hydrocortisone, glucagon, and epinephrine were administered concurrently, they interacted synergistically to produce an amplified and sustained increase in the blood glucose level that was different from the effect of any of the hormones given singly or in pairs.[13] Based on those observations, we administered a similar triple-hormone mixture to normal volunteers to produce sustained physiologic elevations of hormone concentrations over a 3-day period.[14] This resulted in metabolic alterations similar to those observed in patients, including an increased metabolic rate, glucose intolerance, insulin resistance, sodium retention, and potassium wasting. In addition, the infusion resulted in increased negative nitrogen balance, increased whole-body protein breakdown, and a reduction in intracellular muscle glutamine concentrations.[15] The nitrogen loss, however, was modest and was not associated with accelerated net skeletal muscle protein breakdown as indicated by efflux of amino acids from the forearm.[15]

Other hormones might also influence the catabolism of critical illness. Insulin is a major anabolic hormone. It promotes amino acid uptake by muscle[12,16] and reduces protein breakdown.[17] It generally counteracts the effects of corticosteroids.[18] In stable catabolic patients, insulin concentrations are not low, and insulin elaboration by the pancreatic beta cell in response to a glucose challenge is abundant.[19] Early after an injury or in the course of critical illness, however, insulin is commonly suppressed.[20] This early phase of convalescence, called the ebb phase,[21] is often associated with hypovolemia, low perfusion, and enhanced sympathetic activity. Although the concentrations of counterregulatory hormones increase rapidly after an injury, insulin is suppressed until resuscitation is complete. Thus, the relationship between insulin and the three "stress" hormones changes over time.

When healthy human subjects received an infusion of three catabolic hormones, increased net skeletal muscle protein breakdown did not develop.[15] The infusion increased plasma concentrations of the so-called stress hormones, and this was associated with an increase in basal insulin concentration and insulin elaboration in response to carbohydrate.[14] The aim of the current study was to try to reproduce the endocrine alterations that occur both early after the onset of critical illness (ebb phase) and later during convalescence (flow phase). We administered a somatostatin analogue to normal human subjects during the first day of a 3-day infusion of catabolic hormones. This suppressed insulin elaboration temporarily, increased nitrogen loss, and accelerated net skeletal muscle protein breakdown.

MATERIALS AND METHODS

Subjects

Seven normal volunteers (three women and four men) participated in these studies. None of the subjects had evidence of cardiac, pulmonary, renal, or hepatic disease or diabetes. All were vigorous and active. One woman was receiving oral contraceptives, and one man smoked cigarettes occasionally before and after but not during the study protocol. The studies were conducted in the General Clinical Research Center of Washington University School of Medicine, St. Louis, Missouri, between March 1992 and February 1993. Each subject was studied twice. We allowed at least 3 weeks between studies of the same individual, and we tried to study each woman at the same point in her menstrual cycle.

Study Design

The study protocols were reviewed and approved by the Human Subjects Committee of the Washington University School of Medicine. The study procedures, risks, and discomforts were discussed on at least two occasions with each subject before obtaining written informed consent. The subjects were admitted in the afternoon and familiarized with the study procedures and apparatus. They consumed supper and a snack, providing 10 kcal/kg and 1.3 g/kg of protein. This meal was prepared in the kitchen of the Clinical Research Center from standard food items. After 8 P.M., the subject's oral intake was restricted to water only until approximately 5:30

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Table 1. DIET COMPOSITION*

	Day 1	Days 2 and 3
Total energy (kcal)	766 ± 50	2152 ± 172
Carbohydrates (g)	114 ± 8	311 ± 27
Fat (g)	24 ± 1	69 ± 4
Protein (g)	25 ± 2	72 ± 6
Nitrogen (g)	3.9 ± 0.3	11.4 ± 0.9
Sodium (mEq)	25 ± 4	86 ± 8
Potassium (mEq)	43 ± 5	100 ± 9

* Mean ± SEM; n = 7.

P.M. the next day when supper and a snack were consumed. During the next 2 days, the subjects received a standard diet, offered as three meals and a snack. This diet provided a total of 30 kcal/kg and 1 g/kg of protein (Table 1). No food was consumed between 8 P.M. and 8 A.M. each day.

On the morning after admission, basal vital signs were obtained, and the subjects were weighed. After voiding, the subjects rested quietly for at least 30 minutes before indirect calorimetry was performed. An intravenous catheter was placed in a distal forearm vein using 1% lidocaine for local anesthesia. The subjects rested quietly for at least 30 minutes before blood was drawn through the intravenous cannula without stasis for baseline serum chemical analysis, complete blood count (CBC), and hormone concentrations. Both the triple-hormone mixture and a second infusion consisting of either the somatostatin analogue, octreotide (SMS study), or saline (control study) were then begun. For the first 24 hours, the subjects remained in bed, except for bathroom privileges. All urine was collected for subsequent nitrogen analysis. Approximately 8 hours after the start of the study infusions, blood was drawn by venipuncture for levels of glucose and insulin and a CBC. Blood was again drawn for glucose and insulin concentrations 2 hours after supper.

On the following morning, basal vital signs were obtained, and the subjects were weighed. After voiding, they rested for at least 30 minutes before indirect calorimetry was done. Two additional intravenous catheters were placed using local anesthesia. One was passed retrograde into a deep antecubital vein of the forearm, contralateral to the infusion catheter, for sampling of deep venous blood. The other catheter was placed in a dorsal hand vein on the same extremity as the infusion catheter. The hand was wrapped in a heating pad, and the catheter was used to sample arterialized venous blood.[22] After a period of rest, blood was drawn from the deep forearm vein for resting serum chemical analysis, CBC, and hormone concentrations. Two determinations of forearm blood flow and substrate exchange were made at least 30 minutes apart. The octreotide or saline infusion was then stopped, but the triple-hormone infusion was continued for another 48 hours. After removing the intravenous cannulas placed for the forearm studies, the subjects were given breakfast.

For the next 48 hours, the subjects consumed a controlled diet as described and confined their activity to the Clinical Research Center. Fasting serum chemical analysis, CBC, and hormone concentrations were determined, and indirect calorimetry was performed 24 hours later, after 48 hours of infusion. After 3 days of hormone infusion, additional intravenous catheters were placed as before, and duplicate determinations of forearm blood flow and substrate exchange were made. Then, all infusions were stopped, and the intravenous catheters were removed. The subjects were offered a meal of their choice and discharged.

Study Infusions

Triple-Hormone Infusion

The triple-hormone mixture used in this study was similar to that used in previous studies.[14,15,23,24] Hydrocortisone sodium succinate 98 mg (Abbott Laboratories, North Chicago, IL), glucagon 0.13 mg (Eli Lilly, Indianapolis, IN), and epinephrine 1.3 mg (Abbott) were added to 500 mL of normal saline, which also contained 6.25 g of albumin to bind glucagon and 500 mg of ascorbic acid to reduce the hydrolysis of epinephrine. Several 500-mL bags of the mixture were prepared at a time and stored refrigerated until used. The infusion mixture was changed at least every 8 hours, and no container was stored for more than 32 hours before being infused.

Control Infusion

Albumin 1.5 g was added to 100 mL of normal saline. The mixtures were stored refrigerated until used, and a fresh container was hung at least every 8 hours.

Octreotide Infusion

We added 100 μg of the somatostatin analogue, octreotide,* to 100 mL of normal saline containing 1.5 g of albumin. These mixtures were stored refrigerated until used, and a fresh container was hung at least every 8 hours.

* A portion of the octreotide used in this study was generously provided by Richard L. Elton, Ph.D., Director, Neuroendocrine Department, Sandoz Research Institute (East Hanover, NJ).

Infusion Rates

The triple-hormone mixture was infused at a rate of 0.714 mL/kg/min, based on the subject's weight on the first morning of the study. At that rate, the hormone doses were hydrocortisone, 2.3 μg/kg/min; glucagon, 3 ng/kg/min; and epinephrine, 30 ng/kg/min. The control and octreotide mixtures were infused at a rate of 0.15 mL/kg/min, resulting in an octreotide dose of 0.005 μg/kg/min.

Metabolic Studies

Indirect Calorimetry

Indirect calorimetry was performed between 6:30 and 7:00 A.M. each morning of the study, before any manipulations or dietary intake. The subjects were awakened for vital sign measurements at 6:00 A.M. After voiding, they rested for at least 30 minutes before each determination. All measurements were made with the subject reclining comfortably in a thermoneutral, quiet, semidarkened environment. Minute ventilation (V), oxygen consumption (VO_2), and carbon dioxide production (VCO_2) were measured using a portable metabolic cart (MRM 6000, Waters Instrument, Rochester, MN). This automated system consists of a closed-loop spirometer with an in-line CO_2 scrubber for the measurement of VO_2 and a turbine volume meter and infrared CO_2 analyzer for the measurement of V and VCO_2. Before each subject's admission, the instrument was tested for leaks. The CO_2 analyzer was calibrated periodically with a gas of known CO_2 content. After application of a nose clip, the subjects breathed through a mouthpiece into a valved one-way circuit. V, VO_2, VCO_2, the respiratory quotient (RQ), and metabolic rate (MR)† were calculated every minute for at least an 8- to 10-minute period. The averages of at least five values after steady state was achieved were taken to be the results of that determination.

Nitrogen Balance

Beginning on the morning after admission, all urine was collected for three consecutive 24-hour periods, 6 A.M. to 6 A.M. The total urinary nitrogen was determined by chemiluminescence (Antek Instruments, Houston, TX). Aliquots of the 24-hour urine samples were also analyzed for urea nitrogen and creatinine by automated enzymatic technique and for ammonia using an ion-specific electrode (Fisher Scientific, Pittsburgh, PA). The total urinary nitrogen and urinary urea nitrogen were corrected for changes in blood urea nitrogen (BUN) by assuming that urea was distributed throughout the total body water, which was taken to be 60% of the body weight. The total nitrogen loss was taken as the sum of the corrected total urinary nitrogen, with an assumed gastrointestinal loss of 1.3 g/day.[26] Nitrogen balance was calculated as the difference between the total nitrogen intake and the total nitrogen loss.

Forearm Blood Flow and Substrate Exchange

These determinations were made after 24 and 72 hours of infusion after the placement of catheters for blood sampling. The subjects rested quietly in a thermoneutral, quiet, semidarkened environment for at least 30 minutes before the first determination. A second determination was made at least 30 minutes after the first. Blood flow in the forearm opposite the infusion catheter was measured by venous occlusion capacitance plethysmography.[27] An infant blood pressure cuff at the wrist was inflated to 250 to 300 mm Hg 2 to 3 minutes before the flow measurement to exclude blood flow through the hand. A pressure cuff around the upper arm was inflated intermittently to 55 to 60 mm Hg. This pressure consistently occluded venous outflow and resulted in a maximum rate of forearm swelling, which was used to calculate forearm blood flow. Three to five measurements were made, and the mean was calculated. Immediately before blood flow measurement, deep venous and arterialized venous blood samples were drawn simultaneously for the determination of glucose and whole-blood amino acid concentrations. Arterialized venous minus deep venous concentration differences were subsequently calculated and multiplied by the flow to determine the forearm glucose and amino acid exchange.

The plasma glucose level was determined in both arterialized venous and deep venous blood using a Glucose-II analyzer (Beckman Instruments, Fullerton, CA). Although the whole-blood glucose concentration is lower than the plasma value, in previous studies, we found that the arterialized venous minus deep venous glucose concentration difference was similar if calculated on the basis of either whole-blood or plasma values.[28]

Aliquots of arterialized venous and deep venous whole blood were deproteinized with 10% perchloric acid. The protein-free filtrates were mixed with 0.2 mmol/L sodium acetate buffer, adjusted to a pH of 4.8 to 4.85, diluted to volume, and stored at −70 C until analyzed. Amino acid concentrations were determined using reverse-phase, high-performance liquid chromatography (Pico-Tag, Waters Chromatography, Milford, MA). The thawed samples were derivatized with phenylisothiocyanate (Pierce-Rockford, Rockford, IL), lyophilized, and reconstituted. We injected 20 μL of the reconsti-

† The MR was calculated using the Weir equation[25] and setting the nitrogen term to zero as follows: MR (in kilocalories per 24 hours) = {[3.94 × VO_2 (in reciprocal minutes)] + [1.11 × VCO_2 (in reciprocal minutes)]} × 1440 (in minutes per 24 hours).

tuted sample into a 3.9-mm × 30-cm reverse-phase silica column maintained at 46 C in a heat block. A computer-controlled gradient elution using two eluants (98% water:2% acetonitrile and 15% methanol:45% acetonitrile:40% water, Waters Chromatography) permitted separation of all the amino acids found in protein. The phenylthiocarbamyl amino acid derivatives were detected by ultraviolet absorption at 254 nm. This method is reported to have a sensitivity of 1 pmol/μL of reconstituted sample. The amino acid concentrations were quantified from the areas of the individual peaks related to an internal standard. A solution of acidic and basic amino acid standards (Sigma Chemical, St. Louis, MO) to which a known quantity of L-glutamine (Sigma) was added was assayed with each rum of unknowns and served as an external standard. Methionine sulfone (Sigma) was added to each external standard and unknown sample as an internal standard. In addition, an aliquot from a pool of protein-free filtrates of blood from normal individuals was included with each run to determine interassay variability.

Other Analytic Techniques

Blood was drawn under resting conditions each morning for levels of potassium, BUN, and creatinine and CBC by standard laboratory methods. Radioimmunoassay techniques were used to quantify both circulating concentrations of cortisol (Corning Medical Diagnostics, Medfield, MA), glucagon,[29] and insulin[30] and the urinary concentration of C-peptide (Immunex, San Diego, CA). The plasma epinephrine level was measured radioenzymatically.[31] Plasma-free fatty acid concentrations were determined enzymatically.[32]

Calculations

Statistical calculations were performed using PC SAS Statistical Software (SAS Institute, Cary, NC). All results are expressed as the mean ± SEM. A paired Student's t test was used to test for differences between the group means. Because of the variability inherent in the determination of forearm blood flow and glucose and amino acid exchange, these data could not be reliably paired. Thus, all these determinations (n = 14 per group) were pooled and tested for differences by unpaired t tests. Differences were accepted as significant for $p < 0.05$.

RESULTS

Each subject participated in both studies (SMS and control). During one study (control group), the triple-hormone mixture was infused continuously for 3 days, and an additional saline solution was infused for the first 24 hours. During the other study (SMS group), the somatostatin analogue, octreotide, was administered during the first 24 hours of a 3-day triple-hormone infusion. The order of the studies was varied. The initial weights were comparable between the two study periods (Table 2).

Table 2. CHARACTERISTICS OF STUDY SUBJECTS

Subject	Sex	Age (yr)	Height (cm)	First Morning Weight Control (kg)	First Morning Weight SMS (kg)
A	F	22	162	61.3	61.1
B	F	20	175	60.6	59
C	M	20	184	104	103.7
D	M	30	168	65.1	64
E	F	22	174	73.5	74.2
F	M	24	170	69.3	66
G	M	22	174	68	72.3
Mean ± SEM (n = 7)		23 ± 1	172 ± 2	71.7 ± 5.2	71.5 ± 5.3

The subjects tolerated all infusions and study procedures well. As observed in previous studies, the subjects variously reported consciousness of the heart beat, fine tremors, and reduced ability to concentrate. The resting heart rate increased and blood pressure widened during the hormone infusion. On two occasions, mild phlebitis developed, and a new infusion catheter was placed in the opposite arm. During the octreotide infusion, two subjects complained of mild nausea while eating supper. One subject had cramps and passed a loose stool approximately 15 hours after the octreotide infusion was stopped. Otherwise, there were no complications related to the infusions or study procedures.

Laboratory Data

The circulating concentrations of cortisol, glucagon, and epinephrine were increased throughout the triple-hormone infusion (Table 3). The octreotide infusion did not appreciably modify these increases. By paired t tests, the concentration of epinephrine after 48 hours was lower than the control value during the SMS studies, but the mean epinephrine concentrations for the entire 3-day infusion were similar in the two studies (616 ± 30 pg/mL in the control study *vs.* 535 ± 73 pg/mL in the SMS study, $p = 0.18$).

The hormone infusion induced a sustained leukocytosis, similar to previous observations,[14] and an increase in platelet count. These effects were not influenced by octreotide. During both studies, the subjects demonstrated a comparable reduction in concentrations of hemoglobin, BUN, creatinine, and potassium. The decrease in

Table 3. LABORATORY DATA

	Hours of Infusion				
	0	8	24	48	72
Control					
Cortisol (μd/dL)	19.4 ± 4.4		45.3 ± 6.6	40.8 ± 8.0	41.0 ± 6.4
Glucagon (pg/mL)	155 ± 14		269 ± 25	265 ± 21	286 ± 21
Epinephrine (pg/mL)	46 ± 7		570 ± 50	631 ± 56	653 ± 83
BUN (mg/dL)	12 ± 1		8 ± 1	9 ± 1	9 ± 1
Creatinine (mg/dL)	0.9 ± 0.1		0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.1
Potassium (mEq/L)	4.1 ± 0.1		3.8 ± 0.1	4.1 ± 0.1	3.7 ± 0.1
Hemoglobin (g/dL)	13.3 ± 0.6	13.4 ± 0.6	12.7 ± 0.5	12.9 ± 0.5	12.4 ± 0.5
WBC × $10^3/mm^3$	5.6 ± 0.4	13.2 ± 0.9	15.9 ± 1.3	16.7 ± 1.5	267 ± 23
Platelet × $10^3/mm^3$	212 ± 210	267 ± 17	263 ± 12	265 ± 24	267 ± 23
SMS					
Cortisol (μg/dL)	18.4 ± 3.7		44.2 ± 6.8	45.3 ± 6.7	43.4 ± 7.3
Glucagon (pg/mL)	152 ± 20		293 ± 19	277 ± 27	289 ± 13
Epinephrine (pg/mL)	36 ± 8		525 ± 113	463 ± 57*	615 ± 122
BUN (mg/dL)	13 ± 1		11 ± 1	11 ± 1	10 ± 1
Creatinine (mg/dL)	0.9 ± 0.0		0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
Potassium (mEq/L)	4.1 ± 0.1		4.0 ± 0.1	3.7 ± 0.1	3.7 ± 0.1
Hemoglobin (g/dL)	13.8 ± 0.6	13.6 ± 0.6	12.6 ± 0.5	12.2 ± 0.7	12.4 ± 0.5
WBC × $10^3/mm^3$	6.0 ± 0.6	12.2 ± 1.2	16.2 ± 1.5	17.9 ± 2.4	13.9 ± 1.1
Platelets × $10^3/mm^3$	207 ± 18	287 ± 19	264 ± 15	267 ± 13	240 ± 27

Mean ± SEM; n = 7.
* $p < 0.05$ compared with control.

BUN concentration during the first 24 hours was greater during the control study than during the SMS one ($p < 0.05$). Free fatty acid concentrations were similar in both studies.

Glucose and Insulin

Each subject had persistent hyperglycemia in both studies (Table 4, Fig. 1). During the SMS study, the glucose level was significantly greater than the control values at 8 hours and 2 hours after the subjects had eaten supper. On the following morning, after 24 hours of infusion, the glucose concentrations remained elevated in both studies but were not statistically different. Peripheral concentrations of insulin were also increased from the basal level during both study periods. After 8 hours of infusion, the insulin concentration appeared to be less during the SMS than the control study, but this difference was not statistically significant. The insulin response to a glucose load, however, appeared markedly suppressed during the SMS study. Two hours after supper during the SMS study, the insulin level was one fourth of the concentration achieved during the control study. After 24 hours of infusion, the insulin concentration was lower in the SMS study than in the control study. The urinary glucose loss during the first 24 hours of infusion was more than twice as great during the SMS study as during the control study (Table 5). Measurements of 24-hour urinary C-peptide excretion were completed for four subjects. The mean value during the octreotide infusion was less than the value during the control study. After 48 hours of triple-hormone infusion, the insulin concentrations were similar in both studies, but the glucose concentration was lower after the octreotide SMS infusion than after the control one. The urinary glucose excretion during the second 24-hour period of the study was lower after the octreotide infusion than was the control level, and C-peptide excretion also appeared to be lower. After 72 hours of infusion, glucose and insulin levels were similar in both studies, and urinary glucose and C-peptide excretion during the third 24-hour period were similar in both studies and were comparable to values observed in previous studies.[14]

Indirect Calorimetry

All subjects were hypermetabolic during the administration of the hormone infusion. The basal values of VO_2 and those obtained during the control studies in which the triple-hormone mixture was infused alone were similar to the results reported previously during an almost identical study protocol.[14,23] The V and VCO_2 values,

Table 4. GLUCOSE, INSULIN, AND FREE FATTY ACID DATA

	Hours of Infusion					
	0	8	11 (2-hr PC)	24	48	72
Control						
Glucose (mg/dL)	93 ± 3	167 ± 8	254 ± 10	149 ± 8	146 ± 6	133 ± 5
Insulin (μU/mL)	5 ± 0	16 ± 2	82 ± 8	19 ± 2	17 ± 2	15 ± 2
FFA (mEq/L)	398 ± 62		89 ± 9	708 ± 19	563 ± 61	572 ± 39
SMS						
Glucose (mg/dL)	90 ± 1	230 ± 10†	286 ± 6†	163 ± 9	139 ± 4*	131 ± 5
Insulin (μU/mL)	5 ± 0	13 ± 2	21 ± 3†	12 ± 1*	15 ± 1	13 ± 1
FFA (mEq/L)	368 ± 87		112 ± 31	572 ± 74	497 ± 59	572 ± 55

FFA: free fatty acids.
Mean ± SEM; n = 7.
* $p < 0.05$.
† $p < 0.01$ compared with control.

however, were consistently lower than those previously observed and yielded unrealistically low values for RQ (RQ < 0.7). Thus, all measured values of VCO_2 were increased by 18%, and new values for RQ and MR were calculated. This affected the values depicted for VCO_2, RQ, and MR (Table 6) but did not affect the statistical relationship between group means.

Twenty-four hours after the start of the infusions, VO_2 was lower when octreotide was administered concomitantly than during the control study (Table 6). The production of CO_2 at 24 hours was not different between the two study protocols. Thus, RQ was higher, and the MR was lower during the SMS study than during the control one. All values at 48 and 72 hours were similar between groups. At 72 hours, the MRs were 16% and 14% above basal values during the control and SMS studies, respectively. These values were similar to previous observations.

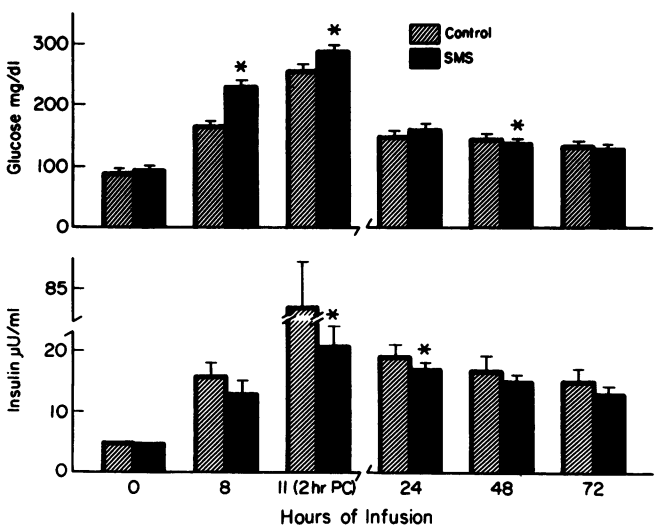

Figure 1. Glucose and insulin concentrations (n = 7). The data are shown as the mean ± SEM. *p < 0.05 compared with control.

Nitrogen Loss and Balance

The subjects had persistently negative nitrogen balance during both study protocols (Table 7). During the first 48 hours of infusion, however, the daily total whole-body nitrogen loss was greater during the SMS study than during control one. Because the nitrogen intake was similar in both study protocols, the nitrogen balance was 2.8 ± 0.6 and 3.0 ± 0.9 g/day more negative, respectively, during the first 2 days of the SMS study than dur-

Table 5. URINARY GLUCOSE AND C-PEPTIDE

	Control	SMS
Glucose (g) (n = 7)		
Day 1	20.9 ± 5.4	43.6 ± 6.5*
Day 2	18.4 ± 5.1	9.9 ± 5.7*
Day 3	14.9 ± 3.0	17.5 ± 5.9
C-Peptide (μg) (n = 4)		
Day 1	101 ± 13	71 ± 13
Day 2	170 ± 32	129 ± 12
Day 3	189 ± 12	188 ± 13

Mean ± SEM.
* $p < 0.05$ compared with control.

Table 6. INDIRECT CALORIMETRY

	Basal		24 Hours		48 Hours		72 Hours	
	Control	SMS	Control	SMS	Control	SMS	Control	SMS
Minute ventilation (L/min)	3.59 ± 0.16	3.49 ± .21	4.15 ± 0.18	4.31 ± 0.17	4.19 ± 0.20	4.14 ± 0.29	4.03 ± 0.21	3.94 ± .22
O_2 consumption (mL/min)	215 ± 17	212 ± 8	287 ± 18	267 ± 18†	259 ± 15	258 ± 22	254 ± 21	249 ± 22
CO_2 production (mL/min)§	193 ± 13	190 ± 10	217 ± 11	232 ± 13	217 ± 14	214 ± 10	214 ± 14	204 ± 10
Respiratory quotient‡	.91 ± .05	.90 ± .03	.76 ± .02	.87 ± 0.03†	.84 ± .02	.85 ± 0.06	.85 ± .03	.83 ± 0.04
Metabolic rate (kcal/m^2 • hr)‡	34.7 ± 1.6	34.2 ± 0.9	44.9 ± 1.8	42.6 ± 1.4*	41.3 ± 1.5	40.4 ± 1.6	40.4 ± 2.0	38.9 ± 1.7

Mean ± SEM; n = 7.
* $p < 0.05$.
† $p < 0.01$, compared with control.
‡ Calculated using adjusted CO_2 production.
§ Values depicted represent measured values + 18% (see text).

ing the control one (Fig. 2). During the third 24-hour period, the nitrogen loss and balance were similar in both groups. The cumulative nitrogen balance during the SMS study was −24.1 ± 1.5 g/day, which was significantly more negative than during control study (−17.8 ± 2.1 g/day, $p < 0.02$). The increased nitrogen loss during the SMS study reflected an increased loss of both urea and ammonia. Creatinine excretion was similar between groups.

Electrolyte Excretion

Sodium intake was provided in the diet (86 mEq/day) and in the triple-hormone infusion (0.11 mEq/kg/hr or approximately 189 mEq/day). Potassium was provided in the diet only (100 mEq/day). Urinary excretion of sodium and potassium (Table 8) indicated that the subjects retained sodium but had a net negative potassium balance. During the 24-hour octreotide infusion, potassium excretion was greater than the control level. In the same group, sodium excretion on day 3 was greater than the control value. During the first 2 days, sodium excretion appeared to be reduced. The cumulative sodium and potassium excretion were similar in both studies.

Forearm Blood Flow and Glucose and Lactate Exchange

Forearm blood flow was similar during both studies at 24 and 72 hours of hormone infusion (Table 9). Glucose uptake was greater after 24 hours of the SMS study than the control one. There was no difference between groups after 72 hours of hormone infusion. The values for forearm blood flow and glucose uptake were similar to those obtained during similar study protocols.[14]

Amino Acid Concentrations

Based on 34 separate measurements of the amino acid concentrations in the pooled protein-free filtrate, the co-

Table 7. URINARY NITROGEN LOSS (g)

	Total N	Urea N	Creatinine	Ammonia
Control				
Day 1	12.1 ± 1.1	11.0 ± 1.1	1.17 ± 0.2	1.17 ± 0.12
Day 2	14.7 ± 1.0	13.7 ± 1.0	1.8 ± 0.1	1.61 ± 0.14
Day 3	13.6 ± 1.0	12.4 ± 1.2	1.7 ± 0.2	1.65 ± 0.07
Cumulative loss	40.5 ± 3.0	37.1 ± 3.0	5.2 ± 0.4	4.43 ± 0.30
SMS				
Day 1	14.9 ± 0.8*	13.7 ± 0.7*	1.8 ± 0.1	1.24 ± 0.07
Day 2	17.8 ± 0.4*	16.3 ± 0.6*	1.6 ± 0.1	1.93 ± 0.20
Day 3	14.3 ± 0.6	13.4 ± 0.6	1.7 ± 0.1	1.92 ± 0.21
Cumulative loss	46.9 ± 1.4*	40.7 ± 2.0†	5.2 ± 0.3	5.10 ± 0.44

Mean ± SEM; n = 7.
* $p = 0.05$ compared with control.

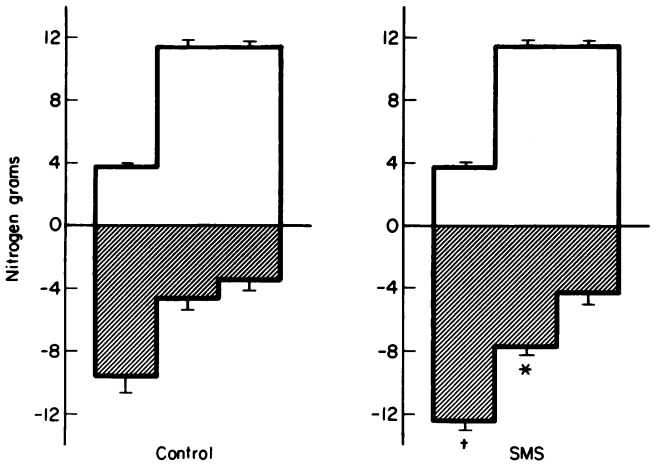

Figure 2. Nitrogen intake and balance (n = 7). The mean ± SEM were calculated assuming a stool nitrogen loss of 1.3 g/day. *p < 0.02 compared with control.

efficients of variation (CV) for the determination of each amino acid could be calculated. Seventeen of the amino acids were determined with CV < 10% (mean CV, 4.92 ± 0.46%). Three amino acids (aspartate, phenylalanine, and cysteine) had a CV between 10% and 20%. The cysteine peak, however, was not interpretable in several instances. Another five amino acids (phosphoserine, hydroxyproline, citrulline, methionine, and tryptophan) could only be determined with a CV > 20%. Because amino acid assays of samples from different studies of the same individual might be performed at different times and the CV of the determination was large in relation to the arteriovenous concentration differences, the five amino acids with a CV > 20% and cysteine were not included in the data analysis. Total amino acid and amino acid nitrogen concentrations were then calculated

Table 8. URINARY ELECTROLYTE EXCRETION

	Control	SMS
Sodium (mEq)		
Day 1	98 ± 18	86 ± 15
Day 2	112 ± 20	109 ± 22
Day 3	134 ± 30	165 ± 29*
Cumulative loss	344 ± 59	359 ± 29
Potassium (mEq)		
Day 1	88 ± 7	113 ± 10*
Day 2	118 ± 9	98 ± 8
Day 3	105 ± 11	110 ± 10
Total	294 ± 32	321 ± 25

Mean ± SEM; n = 7.
* p < 0.015 compared with control.

Table 9. FOREARM BLOOD FLOW AND GLUCOSE UPTAKE

	Control	SMS
24 hours		
Blood flow (mL/100 mL • min)	9.34 ± 0.97	7.26 ± 0.58
Glucose uptake (mg/100 mL • min)	0.06 ± 0.02	0.26 ± 0.09*
72 hours		
Blood flow (mL/100 mL • min)	5.38 ± 0.44	6.41 ± 0.71
Glucose uptake (mg/100 mL • min)	0.13 ± 0.04	0.14 ± 0.04

Mean ± SEM; n = 14 determinations.
* p < 0.05 compared with control.

for each sample by adding the measured concentrations of 19 amino acids and correcting for the amino acids that contained more than one nitrogen atom.

The amino acid concentrations in arterialized venous whole blood are listed in Table 10. After 24 hours of infusion, the total amino acid concentration and total

Table 10. ARTERIAL WHOLE BLOOD AMINO ACID CONCENTRATIONS (μmol/L)

	24 Hours		72 Hours	
	Control	SMS	Control	SMS
Aspartate	96 ± 8	98 ± 9	97 ± 9	94 ± 8
Glutamate	148 ± 5	145 ± 6	158 ± 6	148 ± 6
Serine	83 ± 3	83 ± 3	116 ± 5	101 ± 5
Asparagine	69 ± 4	74 ± 4	92 ± 5	84 ± 4
Glycine	188 ± 5	188 ± 6	216 ± 7	189 ± 5
Glutamine	544 ± 17	577 ± 31	574 ± 20	490 ± 26*
Taurine	335 ± 16	346 ± 18	331 ± 13	313 ± 15
Histidine	97 ± 4	99 ± 5	103 ± 6	97 ± 5
Threonine	80 ± 6	103 ± 6	131 ± 11	131 ± 8
Alanine	158 ± 4	194 ± 18	291 ± 23	224 ± 25
Arginine	37 ± 2	41 ± 3	63 ± 4	50 ± 5
Proline	94 ± 6	108 ± 8	133 ± 7	104 ± 8
Tyrosine	38 ± 2	45 ± 2	50 ± 4	44 ± 4
Valine	134 ± 10	154 ± 7	180 ± 7	161 ± 5
Isoleucine	66 ± 5	71 ± 2	67 ± 4	65 ± 2
Leucine	86 ± 6	102 ± 4	105 ± 4	94 ± 3
Phenylalanine	43 ± 1	46 ± 2	49 ± 2	42 ± 2
Ornithine	38 ± 2	41 ± 3	55 ± 2	46 ± 3
Lysine	83 ± 5	98 ± 6	128 ± 7	108 ± 7
BCAA	286 ± 19	327 ± 13	352 ± 13	320 ± 8
TAA	2419 ± 56	2617 ± 90	2937 ± 115	2587 ± 111*
TAAN	3459 ± 88	3731 ± 143	4180 ± 164	3660 ± 166*

BCAA: branched-chain amino acids; TAA: total amino acids; TAAN: total amino acid nitrogen.
Mean ± SEM; n = 14 determinations.
* p < 0.05.
† p < 0.01 compared with control.

Table 11. FOREARM AMINO ACID EXCHANGE (nmol/100 mL · min)

	24 Hours		72 Hours	
	Control	SMS	Control	SMS
Aspartate	23 ± 10	16 ± 7	20 ± 6	1 ± 9
Glutamate	91 ± 15	63 ± 9	67 ± 9	79 ± 14
Serine	−15 ± 10	−5 ± 10	−20 ± 6	−50 ± 24
Asparagine	−36 ± 10	−15 ± 8	−37 ± 5	−65 ± 13*
Glycine	−76 ± 19	−61 ± 7	−46 ± 8	−90 ± 18*
Glutamine	−326 ± 67	−331 ± 29	−227 ± 24	−344 ± 54
Taurine	−2 ± 36	24 ± 32	−4 ± 13	−9 ± 25
Histidine	21 ± 15	−50 ± 13†	−15 ± 9	−25 ± 9
Threonine	−35 ± 23	−77 ± 15	−58 ± 11	−80 ± 14
Alanine	−120 ± 32	−111 ± 24	−176 ± 20	−282 ± 44*
Arginine	−38 ± 10	−34 ± 6	−47 ± 6	−60 ± 12
Proline	−46 ± 13	−38 ± 10	−49 ± 7	−79 ± 13*
Tyrosine	−18 ± 5	−14 ± 3	−13 ± 2	−28 ± 4†
Valine	−42 ± 16	−26 ± 9	−29 ± 7	−72 ± 15*
Isoleucine	−42 ± 11	−31 ± 3	−14 ± 8	−40 ± 8*
Leucine	−46 ± 11	−41 ± 5	−40 ± 7	−75 ± 12*
Phenylalanine	−18 ± 8	−16 ± 4	−19 ± 3	−29 ± 5
Ornithine	−7 ± 8	5 ± 4	1 ± 4	−13 ± 7
Lysine	−19 ± 16	−13 ± 14	−49 ± 9	−79 ± 15
BCAA	−130 ± 35	−98 ± 13	−83 ± 17	−187 ± 32†
TAA	−751 ± 252	−754 ± 102	−748 ± 85	−1321 ± 194*
TAAN	−1211 ± 360	−1309 ± 147	−1233 ± 126	−2051 ± 296*

BCAA: branched-chain amino acids; TAA: total amino acids; TAAN: total amino acid nitrogen.
Mean ± SEM; n = 14 determinations.
* $p < 0.05$.
† $p < 0.01$ compared with control.

amino acid nitrogen concentration were lower in the SMS study than in the control one. By 72 hours, however, the amino acid concentrations in the control study had generally increased, and the total amino acid and amino acid nitrogen concentrations were then greater than those in the SMS study.

Forearm Amino Acid Exchange

The exchange rates of 19 amino acids across the forearm were calculated and are listed in Table 11. After 24 hours of hormone infusion, the forearm exchange rates of most individual amino acids, total amino acids, and amino acid nitrogen were similar between study groups. After 72 hours of infusion, however, the forearm exchange of total amino acids and amino acid nitrogen was significantly greater in the SMS study than in the control one (Fig. 3). This was caused by an increase in the exchange of most amino acids, including alanine and the branched-chain amino acids (BCAA), leucine, isoleucine, and valine. Glutamine exchange appeared to be greater, but the difference was not statistically significant ($p = 0.059$).

DISCUSSION

The purpose of this study was to characterize further the influence of endocrine alterations on the develop-

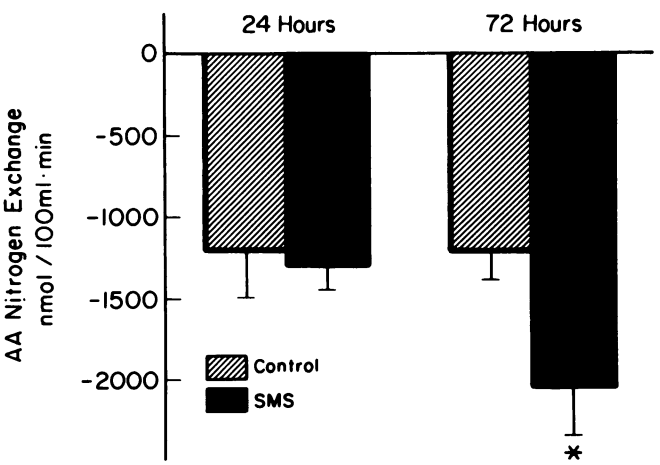

Figure 3. Total amino acid nitrogen exchange across the forearm (n = 14 determinations). The data are shown as the mean ± SEM. *$p < 0.02$ compared with control.

ment of metabolic responses in sick and critically ill patients. We attempted to mimic the changes in stress hormone and insulin concentrations that occur in such patients in normal subjects. We administered a triple-hormone mixture to achieve sustained physiologic elevations of the three stress hormones during a 3-day period. We also infused the somatostatin analogue, octreotide, for the first 24 hours of the triple-hormone infusion. In this way, we hoped to suppress insulin elaboration temporarily when the stress hormone concentrations first increased, which is a common pattern in patients. To simulate the clinical situation still further, we withheld nutrient intake initially, and we required bed rest for the first 24 hours and restricted activity thereafter.

The octreotide infusion (SMS study) temporarily suppressed insulin elaboration in response to the increased glucose concentrations induced by the triple-hormone mixture. The glucose concentrations were greater than the control levels, and the insulin concentrations were less. After supper, there was a marked insulin response in the subjects receiving the triple-hormone infusion alone, but there was a small response during the SMS study. Urinary glucose loss during the first day was greater in the SMS study than in the control one, presumably reflecting the generally higher glucose concentration. C-peptide excretion appeared lower during the SMS study. After the octreotide infusion was stopped, glucose and insulin concentrations and urinary glucose and C-peptide levels appeared to be similar, indicating that the ability to elaborate insulin was largely restored.

Octreotide had little effect on many of the other responses induced by the triple-hormone infusion. Stress hormone concentrations, leukocytosis, thrombocytosis, free fatty acid levels, changes in BUN and creatinine, and cumulative sodium and potassium excretion were all similar in both studies. These results also agreed with values reported previously from similar infusion protocols,[14,24] with the exception of the glucagon concentrations. This discrepancy is thought to be related to recent improvements in the specificity of the antibodies used in the radioimmunoassay.

Alterations in respiratory gas exchange were generally similar in both studies, except on the second morning of the study. Oxygen consumption and the MR were lower after 24 hours of octreotide infusion than in the control study. The difference was small but statistically significant. Carbon dioxide production was affected little; therefore, the RQ was higher during the SMS study than during the control one, reflecting a greater dependence on glucose oxidation. These observations suggest that insulin may contribute to the hypermetabolism induced by hormone infusion.

The suppression of insulin during the first 24 hours of triple-hormone infusion resulted in an increase in urinary nitrogen loss. This increased nitrogen wasting persisted during the second 24 hours after SMS was discontinued. During day 3, the net nitrogen loss was similar in both studies, and the level was similar to earlier observations during day 3 of triple-hormone infusions.[14] Previous studies demonstrated that physiologic elevations of stress hormone concentrations increased the whole-body nitrogen loss by 3 g/day. The studies reported here suggest that temporary suppression of insulin elaboration at the onset of stress hormone elevation results in an additional nitrogen loss of 3 g/day. The magnitude of the nitrogen loss observed in these studies of normal subjects receiving triple-hormone infusion and a short octreotide infusion (14 to 17 g/day) approached that observed in critically ill patients. For example, one group recently performed a meta-analysis of data from a combined group of surgically treated patients receiving either enteral or parenteral nutrition.[33] Mean values for urinary nitrogen excretion were 13.3 and 12.5 g/day for the two types of nutritional support, respectively. Associated with the nitrogen loss was an increased loss of potassium during the first 24 hours, consistent with the loss of intracellular constituents.

Despite the greater whole-body nitrogen loss in the SMS study, the efflux of amino acids from the forearm was not different in the two studies 24 hours after the start of the infusions. However, 48 hours later, when the nitrogen loss in the two studies was similar, the subjects who had received the octreotide infusion demonstrated a 66% increase in forearm amino acid efflux. This appeared to be a generalized response. Significant increases in efflux were demonstrated for several amino acids. The BCAA efflux is thought to be closely related to the total nitrogen flux in muscle.[34,35] The BCAA efflux was doubled in these studies after the octreotide infusion.

Temporary suppression of insulin elaboration at the onset of elevations of stress hormone concentrations led both to increased nitrogen wasting and to accelerated net skeletal muscle protein breakdown. Most of the extra nitrogen loss occurred during the first 48 hours of infusion, at a time when there was no appreciable difference in forearm amino acid nitrogen efflux between the two studies. This suggests that enhanced mobilization of amino acids from peripheral tissue does not serve as the source for the extra nitrogen loss. Presumably, splanchnic tissues is the source. When hypoglycemia was induced with an insulin infusion and whole-body leucine kinetics and interorgan amino acid exchange were measured, an increase in protein turnover and oxidation and more than a fourfold increase in the net release of leucine and other amino acids across the gut were observed.[36] These dynamic changes were associated with increased

stress hormone concentrations. When euglycemia was maintained during the insulin infusion, there were no elevations of catabolic hormone concentrations and no change in the gut balance of leucine. Interactions between insulin and the stress hormones may thus affect the splanchnic amino acid balance. Glucagon may be particularly important in this regard because it may enhance hepatic urea production[37] and increase hepatic proteolysis.[38] Insulin, however, appears to be effective in blunting these proteolytic effects.[39,40] Our subjects were not hypoglycemic, but they had increased concentrations of stress hormones, including glucagon. The higher insulin concentrations observed when the triple-hormone mixture was infused alone may have blunted this catabolic glucagon effect.

The accelerated efflux of amino acids from the forearm, which developed after the 24-hour octreotide infusion was stopped, may reflect a primary effect of the hormonal environment on muscle protein metabolism. Glucocorticoids both accelerate the breakdown of protein in muscle, especially myofibrillar proteins, and reduce protein synthesis.[11] Reduced concentrations of insulin exaggerate the proteolytic effect.[41] The effect of cortisol and insulin on skeletal muscle protein metabolism was studied *in vitro*.[42] Insulin blocked the proteolytic effect of cortisol when it was included in the incubation medium. When the muscle had been preincubated with cortisol alone, however, insulin attenuated, but did not completely suppress, the increased proteolysis. Thus, the suppression of insulin during the first 24 hours of the triple-hormone infusion, when hypercortisolemia was achieved and sustained, may have permitted the muscle proteolytic effect of cortisol to develop. It may have been blunted somewhat at 24 hours by a relative increase in the total amino acid concentration[43] and in forearm glucose uptake. Alternatively, the increased forearm amino acid efflux observed at 72 hours might reflect a secondary mobilization of peripheral amino acids to restore the protein and amino acid pools mobilized during the octreotide infusion. The observation that amino acid concentrations increased between 24 and 72 hours in the control studies was consistent with this interpretation. The lower amino acid concentrations at 72 hours in the SMS study may have contributed to the increased amino acid release observed.[43]

The catabolic hormonal effects of the stress hormones, the opposing effects of insulin and possibly other anabolic hormones, and the temporal relationship between them appear to be responsible in part for the accelerated net breakdown of skeletal muscle that occurs in critically ill patients. These debilitating effects may be further influenced by food deprivation and inactivity.[44,45] In addition, certain features of critical illness, such as acidosis[46] and ischemia–reperfusion injury, could exacerbate the cellular responses.

Injured and critically ill patients typically have a wound or focus of inflammation. Recent interest has focused on the influence of a variety of inflammatory mediators, cytokines, on the catabolic responses to critical illness. Early reports suggested that some of these might be responsible for the increased muscle proteolysis,[47,48] but later studies found no such effect.[49] One group studied the metabolic effects of tumor necrosis factor (TNF) in humans.[50] TNF caused anorexia, and the decreased food intake appeared to be entirely responsible for the increased net nitrogen loss observed. Further studies in humans suggested that inflammatory and metabolic mediators had different influences and that both were involved in the clinical expression of critical illness.[51] The elaboration of TNF and a cascade of other cytokines in response to an inflammatory stimulus, such as endotoxin, may initiate a variety of dramatic catabolic clinical events, including death,[52–54] but it usually also leads to increased stress hormone concentrations.[52] The proteolysis in muscle associated with TNF and sepsis is dependent on the presence of glucocorticoids.[55–58]

In this study, we attempted to characterize further the influence of the endocrine environment on the metabolic responses associated with injury and critical illness. We specifically investigated how insulin might affect catabolic responses and attempted to simulate the pattern of insulin elaboration observed in patients by administering a somatostatin analogue to suppress insulin elaboration during the first 24 hours of a 3-day infusion of stress hormones. As in previous studies, hypermetabolism developed with increased nitrogen loss, glucose intolerance, and insulin resistance. When insulin was suppressed temporarily, the subjects had an accentuated nitrogen loss, and there was increased net skeletal muscle protein breakdown. Endocrine changes that occur during critical illness—in both the ebb and flow phases—may have both short- and long-term effects on catabolic responses. Thus, early metabolic intervention may limit the catabolism associated with trauma and critical surgical illness.

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Discussion

DR. PAUL R. SCHLOERB (Kansas City, Kansas): This paper really represents a metabolic dissection of the endocrine influences associated with the metabolic response to injury and critical illness. Actually, it represents an extension of work Dr. Bessey presented before this Association about 9 years ago. Now he's fine-tuned it to illustrate the important role that insulin has to play in this overall metabolic response.

A couple of questions. How did you relate the effect of bedrest to the overall nitrogen balance data? Dr. Bessey has a tremendous amount of information in this manuscript, for which I am grateful. He didn't have time to present it all and it's going to take me many days to absorb it. But among these data he presented glutamine concentrations. I would like, therefore, to ask: What effect did glutamine depletion in muscle have on the overall nitrogen balance results?

A very nice paper. I congratulate you.

DR. DOUGLAS W. WILMORE (Boston, Massachusetts): For a number of years, Dr. Bessey has been interested in recreating surgical illness in normal individuals—not by performing an operation or causing an injury, but rather by infusing the constituents that may regulate the metabolic changes to stress.

The alterations that he has recreated simulate the hypermetabolic response and the hyperglycemia of stress. The one thing that he has failed to recreate has been the increased breakdown of protein in the body. He has reported very slight increases in nitrogen excretion with the administration of cortisol and other counter-regulatory hormones, but he has never totally replicated the accelerated breakdown of skeletal muscle and efflux of amino acids from the extremities that are observed in critically ill patients.

In this study, insulin has been taken away rather than allowed to increase with the triple hormonal infusion. This effect has caused a remarkable change in nitrogen execution. But this study raises two or three interesting questions.

First, is it really the withdrawal of insulin that causes this effect? To unequivocably prove this point, he should give the somatostatin analogue and infuse insulin at the same time. This additional study is necessary because somatostatin blocks other hormonal changes such as growth hormone. Are the effects observed really growth hormone withdrawal? Do you have any other information where you've given insulin along with the somatostatin analogue to convince us that this is truly an insulin effect and not related to other factors? Could we use insulin infusion early on in our surgical patients to reverse the catabolic response?

The second interesting point about the study is that early on patients demonstrate negative nitrogen balance, that is, their liver increases urea generation and we see the urea in the urine. At the same time, the forearm does not release amino acids. Three days later, there is very little urine urea appearing, but at that time point amino acids are coming out of skeletal muscle very rapidly. We appear to be observing a discordant and apparently uncoordinated response.

Are amino acids pulled by the liver or are they pushed out by the muscle? Dr. Bessey, you have examined the data extensively, and would you discuss where the primary effects are occurring? Are the primary effects really occurring on visceral organs and skeletal muscle is responding in a passive manner to visceral organ amino acid uptake, or are primary effects occurring on muscle and causing muscle to release amino acids and these are being pushed into the liver?

This work is the fine-tuning of a metabolic response model. The results provide us with some interesting metabolic opportunities that may allow us to provide better care of our patients. I congratulate Dr. Bessey and his co-workers.

DR. JOSEF E. FISCHER (Cincinnati, Ohio): I'd like to congratulate Dr. Bessey for doing some studies that really are very, very difficult to do in patients, particularly well patients. He raises a number of issues and I suppose it is possible to interpret the data in a slightly different way depending on emphasis.