Protein Synthesis Rates of Skeletal Muscle, Lymphocytes, and Albumin With Stress Hormone Infusion in Healthy Man

M.A. McNurlan, A. Sandgren, K. Hunter, P. Essén, P.J. Garlick, and J. Wernerman

The rate of protein synthesis was assessed in muscle, lymphocytes, and albumin in healthy volunteers administered an infusion of 6.0 μg cortisol + 3.0 ng glucagon + 0.5 nmol epinephrine $min^{-1} \cdot kg^{-1}$. Protein synthesis in muscle tissue was not sensitive to the immediate effects of hormone infusion, but decreased significantly by 18 hours after the infusion had ceased (1.77% ± 0.12% per day *v* 1.29% ± 0.10%, $P < .05$). The rate of protein synthesis in lymphocytes was acutely sensitive to the effect of the hormone infusion, decreasing from 7.15% ± 1.02% per day to 2.47% ± 0.5% ($P < .05$). However, measurements made 18 hours after the end of the hormone infusion indicated that lymphocyte protein synthesis returned to the preinfusion rates. The rate of albumin synthesis was unaltered during infusion of the stress hormones, but was significantly increased when measured 18 hours after ending the hormone infusion (6.84% ± 0.43% per day *v* 7.99% ± 0.45%, $P < .05$). Thus, tissues respond differently to stress hormone infusion, demonstrating the importance of studying multiple organ systems when assessing the regulation of protein metabolism.

PROFOUND LOSS OF BODY protein occurs in clinical conditions such as infection or major trauma.[1] Associated with this loss of body protein are alterations in protein metabolism, including a decrease in most plasma amino acid concentrations with peripheral release of amino acids and uptake by the splanchnic region.[2] Following surgical trauma, we have also demonstrated a rapid and sustained decrease in the rate of protein synthesis in skeletal muscle, which was not reversed by intravenous nutrition.[3]

To understand the mechanism by which the changes seen in trauma are brought about, studies have been conducted involving infusion of the hormones that typically increase in traumatized patients. These stress hormones include cortisol, epinephrine, and glucagon. Infusion of these hormones for 72 hours into healthy subjects results in net protein loss with alterations in carbohydrate metabolism similar to that observed during stressful illness.[4,5] Moreover, Wernerman et al[6] have reported changes in amino acid levels in plasma and muscle tissue with peripheral release and splanchnic uptake of amino acids comparable to that observed following surgery. Changes in muscle ribosome number and distribution consistent with a decrease in the rate of muscle protein synthesis have also been observed[7] in healthy subjects infused with stress hormones for 6 hours. In the present study, we have therefore assessed the rate of muscle protein synthesis directly from the incorporation of labeled phenylalanine into protein. The label was administered as a large (ie, flooding) amount to facilitate quantitation of the isotopic enrichment of the precursor for muscle protein synthesis.[8-13]

However, the responses to injury are not restricted to muscle, so this study has been extended to include effects on protein synthesis rates in peripheral blood lymphocytes and serum albumin, as a means of assessing the impact of stress hormones on the immune system and on the liver. In addition, since trauma has both immediate and prolonged effects, the study examined both the immediate effect of infusion of cortisol, epinephrine, and glucagon into healthy subjects and the effects on protein metabolism in the three organ systems that persist 18 hours after cessation of the hormone infusion. A preliminary report of this study has been presented in abstract form.[14]

SUBJECTS AND METHODS

Subjects and Protocols

Three separate protocols were used to investigate the effect of stress hormone infusion in normal healthy male subjects on the rate of protein synthesis in muscle tissue, peripheral blood lymphocytes, and serum albumin. All three protocols involved intravenous infusion (into fasting subjects) of 0.5 nmol epinephrine (ACO Läkemedel, Solna, Sweden) + 6.0 μg cortisol (Solu Cortef; Upjohn, Puurs, Belgium) + 3.0 ng glucagon (Novo Nordisk, Bagsværd, Denmark) $min^{-1} \cdot kg^{-1}$ for a period of 6 hours. During this time, the subjects reclined on a bed and continued to fast. In experiments 1 and 2, control subjects received an intravenous infusion of saline (1.1 mL/kg/h) for 6 hours.

Measurements of muscle protein synthesis were made from the incorporation of L-[$^2H_5$,ring]phenylalanine administered intravenously at 45 $mg \cdot kg^{-1}$, with increasing enrichment for each successive injection in the same subject beginning with 7.5 atoms% and increasing to 15 atoms% (for subjects studied twice) and 30 atoms% (for subjects studied three times). Unlabeled phenylalanine was purchased from Ajinomoto (Tokyo, Japan) and [$^2H_5$]phenylalanine from MassTrace (Woburn, MA). The solutions of phenylalanine were administered as 2% (wt/vol) solutions and injected into a forearm vein over 10 minutes. Blood samples were taken from a contralateral vein at time points up to 90 minutes after the injection of isotope. At 90 minutes, a percutaneous biopsy of approximately 50 mg was removed from the vastus lateralis and frozen in liquid nitrogen. Also at 90 minutes, a 20-mL blood sample was taken for isolation of peripheral blood lymphocytes.

Experiment 1. Eight control subjects aged 25 ± 1 years (mean ± SEM), 182 ± 3 cm in height, and weighing 72 ± 2 kg were

From The Rowett Institute, Aberdeen, Scotland; the Department of Surgery, State University of New York at Stony Brook, Stony Brook, NY; and the Departments of Anesthesiology at St. Görans Hospital and Huddinge Hospital, Stockholm, Sweden.

Submitted January 2, 1996; accepted May 20, 1996.

Supported by The Scottish Office Agriculture and Fisheries Department, Nestec Inc (Lausanne, Switzerland), the Swedish Medical Research Council (project 04210), and the Maud and Birger Gustavsson Foundation.

Address reprint requests to M.A. McNurlan, PhD, Department of Surgery, SUNY-Stony Brook, Stony Brook, NY 11790-8191.

0026-0495/96/4511-0013$03.00/0

infused with saline for 6 hours after an overnight fast. Eight experimental subjects receiving the 6-hour hormone infusions were aged 24 ± 1 years, 181 ± 1.5 cm in height, and 77 ± 2.5 kg in body weight. Measurements of albumin and muscle protein synthesis were made during the last 1.5 hours of the infusion period.

Experiment 2. Paired measurements of muscle and lymphocyte protein synthesis were made in two groups of six subjects immediately before and during the last 1.5 hours of a 6-hour infusion of either saline or stress hormones. The subjects given saline were aged 22 ± 0.6 years, 183 ± 2 cm in height, and 73 ± 3 kg in weight. The individuals receiving hormone infusion were aged 25 ± 0.6 years, 185 ± 1 cm in height, and 77 ± 3 kg in weight.

Experiment 3. Three successive measurements of muscle protein and albumin synthesis were made in a group of six individuals (age, 27 ± 1 years; height, 180 ± 1.5 cm; weight, 74 ± 1.8 kg). Protein synthesis was measured (1) after an overnight fast, before the hormone infusion; (2) during the last 1.5 hours of the 6-hour hormone infusion; and (3) 24 hours after the start of the hormone infusion, ie, 18 hours after the hormone infusion ended. Measurements of lymphocyte protein synthesis were made (1) before the hormone infusion began and (2) 24 hours after the onset (18 hours after the cessation) of the hormone infusion. Subjects continued to fast during the entire experimental protocol.

Information on the procedures and potential risks was given to each subject before obtaining their written consent. All studies were approved by the Ethics Committee of the Karolinska Institute (Stockholm, Sweden).

Isolation of Peripheral Blood Lymphocytes

Peripheral blood lymphocytes were isolated, using the method of Bøyum,[15] from 20 mL peripheral venous blood in heparinized tubes containing cycloheximide at a concentration of 0.5 mmol/L (Sigma Chemical, St Louis, MO). The blood was diluted 1:1 with saline, layered onto 3 mL density gradients (Lymphoprep; Nycomed, Oslo, Norway) in 5-mL aliquots, and centrifuged at 800 × *g* for 15 minutes at room temperature. Following centrifugation, the lymphocyte layers were removed, washed twice with saline, and stored at −20°C before analysis. Isolation of lymphocytes did not include specific steps to eliminate contamination with macrophages and monocytes.

Albumin Dilution Space

Albumin dilution space was determined from the standard procedure for assessment of plasma volume with ^{125}I-albumin. Measurements of plasma volume were made in experiment 1 only.

Plasma Albumin

The concentration of plasma albumin was determined with bromocresol green as described by Doumas.[16] Isolation of plasma albumin for measurement of enrichment involved precipitation of all plasma proteins with 10% (wt/wt) trichloroacetic acid, with subsequent preferential extraction of albumin with ethanol.[17] Purity of the albumin (Fig 1) was verified with polyacrylamide gel electrophoresis (10% gel) visualized with silver staining.[18]

Plasma Hormones and Metabolites

Plasma glucose was analyzed by a glucose dehydrogenase method.[19] Plasma insulin,[20] glucagon,[21] and cortisol[22] were all determined by radioimmunoassay. Epinephrine and norepinephrine levels were measured by high-performance liquid chromatography with electrochemical detection.[23]

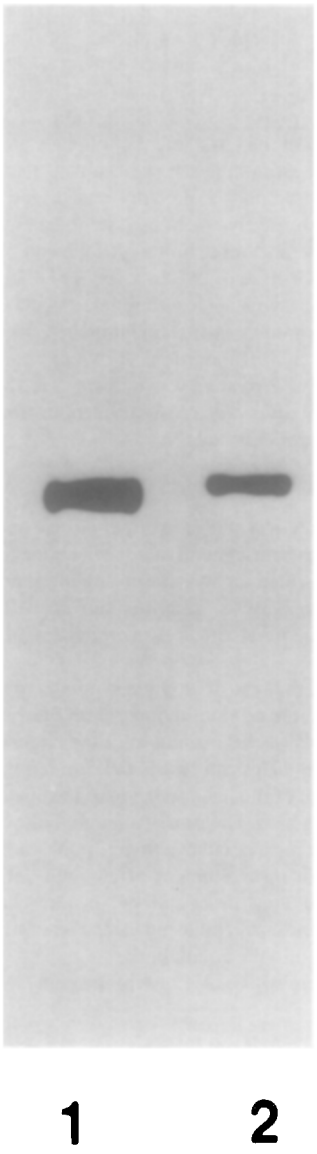

Fig 1. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of albumin isolated from an individual 18 hours after cessation of a stress hormone infusion (lane 1), with standard human albumin (lane 2) for comparison. The gel has been silver-stained.

Protein Synthesis

Measurement of tissue protein synthesis with a flooding dose of amino acids has been described previously for L-[1-^{13}C]leucine in muscle,[10] albumin,[8] and lymphocytes.[13] After the initial studies, the flooding method was modified so that L-[$^{2}H_5$]phenylalanine replaced labeled leucine. This modification, described in detail elsewhere,[12,24] facilitated the measurement of enrichment of the labeled amino acid in protein. The use of L-[$^{2}H_5$]phenylalanine rather than L-[^{13}C]leucine has allowed measurement of the amino acid enrichment in protein by gas chromatography mass spectrometry. This procedure therefore permits measurement of both plasma and protein-derived amino acids with the same instrument, and it also allows a reduction in sample size from approximately 100 mg to as little as 5 mg.

Enrichment of L-[$^{2}H_5$]phenylalanine from plasma was deter-

mined after acid precipitation of protein, followed by cation-exchange chromatography. Ions at m/z 336 and m/z 341 were selectively monitored after electron-impact ionization of the tertiary butyldimethylsilyl derivatives with a VG 12-253 quadrupole mass spectrometer (VG Biotech, Altrincham, UK).

Determination of the enrichment of phenylalanine incorporated into protein was essentially the same for all tissues. Protein was precipitated with 0.2 mol/L perchloric acid, and the precipitates were washed extensively, including solubilization in 0.3 mol/L sodium hydroxide and reprecipitation followed by hydrolysis in 6 mol/L hydrochloric acid. After enzymatic decarboxylation to phenylethylamine (tyrosine decarboxylase; Sigma Chemical, Poole, Dorset, UK) followed by extraction into ether, back-extraction into 0.1 mol/L HCl, and evaporation to dryness, enrichment of the heptafluorobutyryl derivative was determined by monitoring m/z 106 and m/z 109 after electron-impact ionization with the same instrument already described.

Calculations and Statistics

The rate of protein synthesis in either muscle or lymphocytes was calculated from the enrichment of phenylalanine in protein divided by the area described by the plasma phenylalanine enrichment-time curve, as previously described for L-[1-^{13}C]leucine,[10] and expressed as a fractional rate, ie, the proportion of the protein pool renewed per day.

Calculation of the rate of albumin synthesis, described in detail previously,[8] took into account the secretion time, ie, the time delay between synthesis of the protein and appearance of the labeled protein in plasma. This interval is determined experimentally as the zero-intercept of the linear portion of the curve for enrichment in albumin versus time. The rate of albumin synthesis, expressed as a fraction of the intravascular albumin pool, was then calculated from the increase in enrichment of albumin between 50 and 90 minutes and the area described by the enrichment of plasma phenylalanine-time curve, adjusted for the interval between synthesis and secretion into the circulation.

Statistical comparison of rates of protein synthesis between control and treatment groups (experiment 1) was made with Student's t test (unpaired). Rates from successive measurements in the same individuals were evaluated with a paired t test when there were two measurements (experiment 2) and repeated-measures ANOVA when there were three measurements (experiment 3) in the same individual. The data are expressed as the mean ± SEM, and differences were taken to be statistically significant if P was less than .05 (two-tailed).

RESULTS

Intravenous infusion of glucagon + cortisol + epinephrine into healthy subjects produced rapid and sustained elevations in plasma levels of all three hormones. In addition, significant elevations were observed in plasma insulin and glucose. Norepinephrine levels decreased at the beginning of stress hormone infusion, but by the end of the 6-hour infusion the levels were comparable to those in the saline-infused subjects. The data shown in Fig 2 are from experiment 1; similar changes were observed in the other two experiments.

Experiment 1

The rates of muscle protein synthesis from experiment 1 are shown in Table 1. In this experiment, two groups of subjects were investigated: one group received intravenous saline for 6 hours, and one group received intravenous stress hormones. Muscle protein synthesis was measured during the last 1.5 hours of the infusion. The rate of muscle protein synthesis (mean ± SEM) in the saline group was 1.57 ± .075% per day, and in the group that received hormones, 1.48 ± 0.047% per day. These values were not significantly different.

Rates of albumin synthesis in the two groups of subjects are also shown in Table 1. There was no difference in the

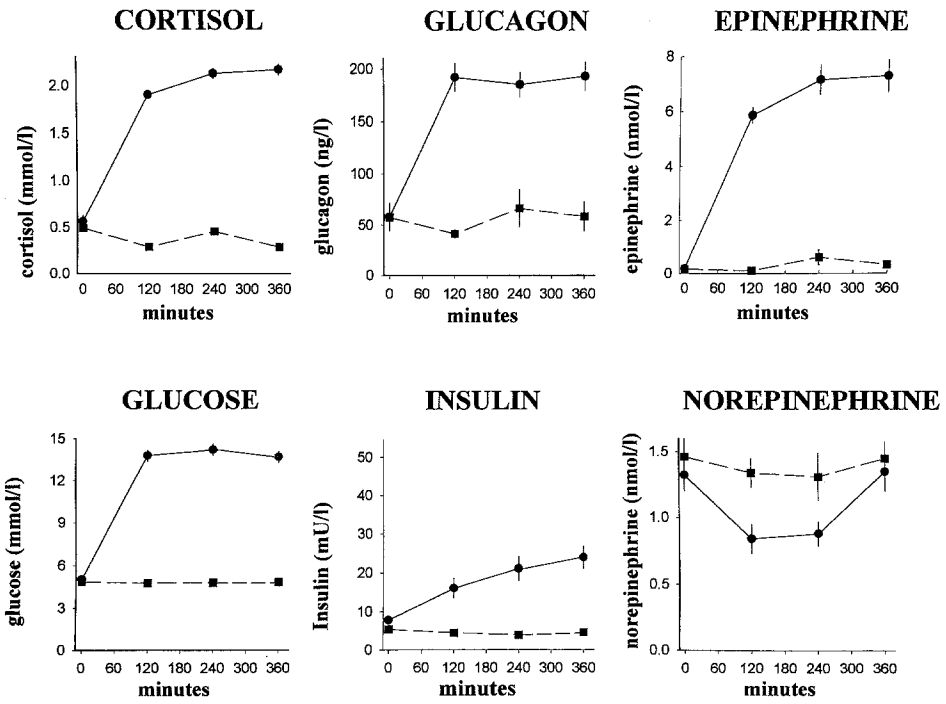

Fig 2. Plasma hormone and glucose concentrations in subjects infused with saline (■) or stress hormones (●). Data are the mean ± SEM; n = 8.

Table 1. Rates of Protein Synthesis in Vastus Lateralis Muscle and Albumin of 16 Healthy Subjects Infused With Saline or Stress Hormones (epinephrine, cortisol, and glucagon) for 6 Hours in Experiment 1 (%/d)

Tissue	Saline	Hormones
Muscle	1.27	1.57
	1.52	1.53
	1.42	1.40
	1.76	1.49
	1.97	1.69
	1.50	1.26
	1.56	1.38
	1.57	1.51
Mean ± SEM	1.57 ± 0.07	1.48 ± 0.05
Albumin	6.43	7.24
	7.20	7.17
	6.53	7.45
	5.00	6.57
	6.63	6.84
	7.89	6.72
	7.66	6.39
	6.08	6.84
Mean ± SEM	6.68 ± 0.33	6.90 ± 0.13

fractional rate of albumin synthesis (control, 6.68 ± 0.33% per day; hormone, 6.90 ± 0.13% per day). The absolute amount of albumin synthesized was also calculated from the fractional synthesis rate and the intravascular albumin mass, derived from the albumin concentration in plasma and the albumin dilution space. The rate of albumin synthesis expressed as the amount of albumin synthesized per day was 9.95 ± 0.48 g in controls and 11.02 ± 0.45 g in hormone-infused subjects. The larger amount of albumin synthesized by the group receiving hormones was due to the higher albumin concentration in this group. The albumin concentration was significantly higher in subjects receiving stress hormones than in control subjects (46.8 ± 0.52 $g \cdot L^{-1}$ *v* 44.7 ± 0.43). However, this was not a result of the hormone infusion, since the difference between these two groups was apparent even before the infusions began (47.1 ± 0.53 *v* 44.7 ± 0.43 $g \cdot L^{-1}$, $P < .01$). Measured plasma volumes were not different between the two groups (3.38 ± 0.2 *v* 3.42 ± 0.13 L). Moreover, there was no difference between the measured plasma volume for all subjects (3.46 ± 0.08 L) and the values predicted from sex, height, and weight (3.40 ± 0.11 L) as suggested by Nadler et al.[25]

L-[$^2H_5$]phenylalanine was injected after 270 minutes of saline or hormone infusion and produced a spike in the concentration of plasma insulin, as we have reported previously.[11] In individuals who were receiving stress hormones, the increase in plasma insulin in response to phenylalanine injection was significantly greater than observed in the saline-infused group.

Experiment 2

In experiment 2, paired measurements of protein synthesis were made before beginning the intravenous infusion of either saline or hormones and then again in the last 1.5 hours of the 6-hour infusion period. The rates of protein synthesis in both muscle and lymphocytes are shown in Table 2. This paired comparison indicated that in muscle the rates of protein synthesis during hormone infusion were slightly but not significantly different from the rates measured before the infusion began. A similar small and nonsignificant decrease occurred in the saline group. The decrease in the hormone group was not significantly larger than in the saline group, but in the two infused groups combined the depression in synthesis was significant ($P < .05$). However, unlike the synthesis rates in muscle, the rate of protein synthesis in peripheral blood lymphocytes was significantly and substantially depressed during infusion of stress hormones. This decrease in the rate of protein synthesis was not observed in individuals receiving saline infusions.

Experiment 3

As in experiment 2, there was a small and nonsignificant decline in the rate of muscle protein synthesis during the last 1.5 hours of hormone infusion (control, 1.77 ± 0.12% per day; hormone, 1.51 ± 0.14% per day). However, rates of muscle protein synthesis were significantly lower when the measurements were made 24 hours after beginning the hormone infusion (1.29 ± 0.10% per day; Table 3). In contrast to the decline observed in muscle, rates of protein synthesis in lymphocytes (Table 3) were not altered 24 hours after initiation of the stress hormone infusion.

Sequential measurements of albumin synthesis in experiment 3 are also shown in Table 3. The measurement made during the last 1.5 hours of hormone infusion was not different from the initial measurement made in the postabsorptive state. However, the measurement made 18 hours after cessation of the hormone infusion indicated that the rate of albumin synthesis had increased by approximately 17%. This increase was not attributable to contamination of isolated albumin with proteins having higher rates of synthesis. Figure 1 depicts the results of sodium dodecyl

Table 2. Rates of Protein Synthesis in Vastus Lateralis Muscle and Peripheral Blood Lymphocytes of Six Healthy Subjects Before (control) and During (infused) Intravenous Infusion of Saline or Stress Hormones (epinephrine, glucagon, and cortisol) in Experiment 2 (%/d)

	Saline		Hormones	
Tissue	Control	Infused	Control	Infused
Muscle	2.00	2.20	2.47	1.94
	1.55	1.37	1.85	1.93
	1.50	1.32	1.49	1.51
	1.37	1.29	1.77	1.23
	2.00	1.29	2.31	1.33
	1.73	1.29	1.76	1.68
Mean ± SEM	1.69 ± 0.11	1.46 ± 0.15	1.94 ± 0.15	1.60 ± 0.12
Lymphocytes	6.51	12.56	6.53	1.07
	—	13.14	11.09	3.18
	12.73	7.83	9.11	—
	7.04	5.34	4.28	3.09
	6.37	13.01	5.60	1.14
	4.98	5.91	6.26	3.87
Mean ± SEM	7.53 ± 1.34	9.63 ± 1.50	7.15 ± 1.02	2.47 ± 0.57*

*Infused *v* control, $P = .02$ (paired *t* test).

Table 3. Rates of Protein Synthesis in Vastus Lateralis Muscle, Peripheral Blood Lymphocytes, and Albumin of Six Healthy Subjects Before (control), During (hormone), and 24 Hours After (postinfusion) Intravenous Infusion of Epinephrine, Glucagon, and Cortisol in Experiment 3 (%/d)

Tissue	Control	Hormone	Postinfusion
Muscle	1.87	1.68	1.32
	1.36	1.53	1.03
	2.06	1.94	1.68
	2.07	1.40	1.19
	1.73	1.57	1.46
	1.52	0.93	1.09
Mean ± SEM	1.77 ± 0.12	1.51 ± 0.14	1.29 ± 0.10*
Lymphocytes	6.52		6.87
	6.67		4.35
	7.08		6.85
	5.14		5.91
	6.49		6.10
	4.22		4.42
Mean ± SEM	6.02 ± 0.45		5.75 ± 1.13
Albumin	7.44	7.90	7.21
	5.86	6.26	8.09
	8.47	7.4	10.08
	6.01	6.32	7.43
	6.03	6.41	8.02
	7.21	7.42	7.1
Mean ± SEM	6.84 ± 0.43	6.95 ± 0.29	7.99 ± 0.45†

*Different from control, $P = .004$ (repeated-measures ANOVA).
†Different from control, $P = .039$ (repeated-measures ANOVA).

sulfate–polyacrylamide gel electrophoresis visualized with silver staining. Despite the deliberate overloading of the gel, there was no evidence of contaminating protein. Moreover, the levels of two typical acute-phase proteins, C-reactive protein and α_1-antitrypsin, were not altered by the infusion of stress hormones.

At the beginning of the third measurement of protein synthesis in experiment 3, ie, 24 hours after beginning the stress hormone infusion, plasma hormone and glucose levels were at or less than preinfusion levels (preinfusion *v* 24 hours later: glucose, 4.9 ± 0.17 *v* 4.5 ± 0.09 mmol/L; insulin, 5.17 ± 0.87 *v* 4.75 ± 0.63 mU/L; glucagon, 101.6 ± 20.10 *v* 99.9 ± 16.3 ng/L; and cortisol, 477.2 ± 37.6 *v* 300.5 ± 54.4 nmol/L). Epinephrine and norepinephrine levels were not measured in this experiment, but with the same protocol in another group of volunteers, epinephrine levels had also returned to normal by 24 hours and norepinephrine levels were not altered (B. Ejesson, C. Lind, E. Vinnars, et al, unpublished results, 1989).

DISCUSSION

The combined infusion of glucagon, cortisol, and epinephrine has been used to study the role of these stress, or counterregulatory, hormones in the mediation of changes in metabolism observed at the tissue level following trauma. Bessey et al[4] have demonstrated that this approach can mimic many of the perturbations observed in trauma, including an elevation in metabolic rate, changes in glucose metabolism such as increased glucose production and decreased glucose disposal, and a loss of body protein manifested as a negative nitrogen balance with increased rates of protein synthesis and degradation. However, studies on the whole body are limited in their ability to distinguish the separate effects on individual tissues, which may change in similar or dissimilar directions. In this study, the differential effects of stress hormone infusion on protein metabolism have been examined in three different tissues: in muscle, which represents the major store of body protein, in peripheral lymphocytes as a measure of the potential effects on the immune system, and in albumin as an indication of the response in liver. Although the tissues investigated are not comprehensive, they do give an indication of the effects in three organ systems.

Protein Synthesis in Skeletal Muscle

In the three experiments of this study, the effect of stress hormone infusion on the rate of protein synthesis in skeletal muscle was small. In experiment 1 (Table 1), there was no difference in the rate of skeletal muscle protein synthesis in the two groups of subjects. The second experiment (Table 2), in which paired measurements were made, showed a decline in the rate of muscle protein synthesis during the last 1.5 hours of stress hormone infusion, but the change was small and not statistically significant. It is of interest that the rate of protein synthesis also declined in subjects who were infused with saline. In the third experiment (Table 3), there was a small decline, similar to that observed in experiment 2, at the end of the hormone infusion, but a larger depression in synthesis was measured 18 hours later. This depression in synthesis was greater than we have observed with 3 days of fasting, which resulted in a depression in synthesis of approximately 15%.[26]

Recent qualitative studies on the subcellular apparatus for muscle protein synthesis have shown a decrease in both the percentage of ribosomes present as polysomes and the ribosome content in response to a 6-hour infusion of stress hormones, both indicative of a decrease in protein synthesis.[27] However, both these data and those for the incorporation of labeled phenylalanine in the present study (Tables 1 to 3) contrast with the 40% stimulation of muscle protein synthesis reported by Gore[28] during a 4-hour infusion of stress hormones. The reason for the difference is not obvious. The protocol of the study by Gore[28] involved infusion of lower levels of epinephrine than this study, 15 versus 91 $ng \cdot kg^{-1} \cdot min^{-1}$. However, although low levels of epinephrine have been shown to be anticatabolic in human skeletal muscle, this occurred through suppression of proteolysis rather than through stimulation of protein synthesis.[29] Moreover, results with the other individual stress hormones show a depression of muscle protein synthesis by corticosteroids in man[30,31] and by glucagon in animals.[32]

Even saline infusion appears to have depressed muscle protein synthesis. Although the difference was not statistically significant, measurements at 6 hours in the saline-infused group of experiment 2 (Table 2) were about 15% lower than the values obtained before infusion. Whether this depression arose from immobility, the continued effects of fasting, or the intravenous saline cannot be discerned from this experiment. It is worth noting that a decline of

approximately 15% in the rate of protein synthesis in vastus lateralis was observed in healthy individuals after 3 days of fasting[26] in which the subjects were not immobilized as they were for the 6-hour intravenous infusions in the present experiment. It would be consistent with the observed data to expect a small decrease in protein synthesis associated with the fasting of these subjects, with only a small depression in muscle protein synthesis being due to the infusion of stress hormones. However, such slight alterations in the rate of muscle protein synthesis with stress hormone infusion suggest that alterations in plasma levels of these hormones are unlikely to account for the substantial reduction in muscle protein synthesis observed within the first few hours after cholecystectomy.[33]

Although they are perhaps not involved in the initial depression in muscle protein synthesis in response to surgical trauma, stress hormones may contribute to the subsequent and persistent effects of trauma. Even transient increases in the concentrations of these stress hormones have effects on muscle that persist even after the hormone concentrations have returned to normal. Ejesson et al[34] have reported that changes in the levels of free amino acids in muscle tissue after cholecystectomy are not apparent immediately postoperatively but become apparent with time after surgery. Similarly, changes in free amino acid levels in muscle were observed with stress hormone infusion in healthy volunteers, persisting even 24 hours after termination of the stress hormone infusion.[34] For some amino acids such as glutamine, the decrease in muscle concentration was small at 6 hours, but was more pronounced ($\sim$20%) 18 hours after cessation of the hormone infusion. These results would suggest that the trauma response observed after surgery is multiphasic and that the initial changes in muscle protein metabolism may not be stress hormone–mediated. However, the subsequent and persistent effects of trauma may be mediated by even transient increases in plasma levels of stress hormones.

Protein Synthesis in Peripheral Blood Lymphocytes

Measurement of the rate of protein synthesis in peripheral blood lymphocytes affords a novel measure of lymphocyte activity in vivo. This assessment differs from in vitro assays that determine the capacity of lymphocytes to respond when tested with strong external stimuli such as mitogens. Rather than testing the capacity for response, the in vivo estimate of protein synthesis is a measure of the metabolic state of lymphocytes within the body. In studies wherein lymphocytes have been activated by injection of interleukin-2, changes in protein synthesis correlate with other parameters of activation such as cell proliferation.[13] In contrast to the observed changes in muscle protein synthesis with stress hormone infusion, protein synthesis in lymphocytes did show an acute sensitivity to the infusion of stress hormones, with a decrease of 60% observable at the end of the 6-hour infusion period (Table 3). This decrease in protein synthesis in vivo is consistent with other studies demonstrating a reduced immunoreactivity with increased levels of cortisol.[35] However, by 18 hours after cessation of the hormone infusion, the rate of protein synthesis in lymphocytes had returned to preinfusion levels (Table 3), suggesting that the inhibitory effect of stress hormones was transient.

Albumin Synthesis

The rate of albumin synthesis, expressed as a percent of the intravascular albumin mass, was similar in saline-infused and stress hormone–infused subjects (experiment 1; Table 1) in whom albumin synthesis was determined during the last 1.5 hours of the 6-hour infusion period. In experiment 3 (Table 3), estimates of albumin synthesis made 18 hours after cessation of the hormone infusion demonstrate a significant elevation of approximately 20% over postabsorptive values. It was not possible to calculate the absolute rates of albumin synthesis in this experiment, since it was not feasible to make three sequential determinations of plasma volume because of the radiation risk associated with radiolabeled albumin. However, there was no observable change in the plasma concentration of albumin (47.3 ± 0.63, 47.8 ± 0.21, and 46.3 ± 0.56 g $\cdot$ L^{-1}), suggesting that plasma volume was not altered by infusion of stress hormones. With no alteration in plasma volume or albumin concentration, the observed increase in the fractional rate of albumin synthesis would be mirrored by a similar increase in the absolute rate. The observed delay in the elevation of albumin synthesis suggests that the effect may not be mediated through a direct hormonal effect on the liver, but that some other mechanism is activated by the stress hormone infusion with persistent effects, such as a change in levels of mRNA for albumin. An increase in albumin synthesis was not observed in perfused liver from rats given infusions of stress hormones, although an elevation in total liver protein synthesis was observed in these animals.[36]

In conclusion, responses to stresses such as injury and infection include complex reactions mediated by a multitude of factors. This study sought to delineate the extent to which elevated plasma levels of epinephrine, glucagon, and cortisol contribute to the overall changes in protein metabolism observed with stress. The study demonstrates that elevation of these hormones has immediate effects on protein metabolism such as a depression in lymphocyte protein synthesis, and effects that develop subsequently, eg, a small depression of muscle protein synthesis and a stimulation of albumin synthesis.

The divergence of effects between muscle, lymphocytes, and liver highlights the importance of assessing the responses of individual tissues. When measurements are made of whole-body protein metabolism, differences in response between tissues cannot be observed. The present study is in accordance with other investigations demonstrating that although infusions of stress hormones can mimic some trauma responses they are insufficient to mimic the rapid and profound responses in protein metabolism observed during trauma.

ACKNOWLEDGMENT

We are grateful to Annette Nilsson for skillful nursing assistance and to Graham Calder for excellent mass spectrometry.

REFERENCES

1. Cuthbertson DP: The metabolic response to injury and its nutritional implications: Retrospect and prospect. JPEN 3:108-129, 1979

2. Gelfand RA, DeFronzo RA, Gusberg R: Metabolic alterations associated with major injury or infection, in Kleinberger G, Deutsch E (eds): New Aspects of Clinical Nutrition. Basel, Switzerland, Karger, 1983, pp 211-239

3. Essen P, McNurlan MA, Sonnenfeld T, et al: Muscle protein synthesis after operation: Effects of intravenous nutrition. Eur J Surg 159:195-200, 1993

4. Bessey PQ, Watters JM, Aoki TT, et al: Combined hormonal infusion simulates the metabolic response to injury. Ann Surg 200:264-281, 1984

5. Gelfand RA, Matthews DE, Bier DM, et al: Role of counterregulatory hormones in the catabolic response to stress. J Clin Invest 74:2238-2248, 1984

6. Wernerman J, Brandt R, Strandell T, et al: The effect of stress hormones on the interorgan flux of amino acids and on the concentration of free amino acids in skeletal muscle. Clin Nutr 4:207-216, 1985

7. Wernerman J, Botta D, Hammarqvist F, et al: Stress hormones given to healthy volunteers alter the concentration and configuration of ribosomes in skeletal muscle, reflecting changes in protein synthesis. Clin Sci 77:611-616, 1989

8. Ballmer PE, McNurlan MA, Milne E, et al: Measurement of albumin synthesis in man: A new approach employing stable isotopes. Am J Physiol 259:E797-E803, 1990

9. Garlick PJ, McNurlan MA, Essén P, et al: Measurement of tissue protein synthesis in vivo: A critical analysis of contrasting methods. Am J Physiol 266:E287-E297, 1994

10. Garlick PJ, Wernerman J, McNurlan MA, et al: Measurement of the rate of protein synthesis in muscle of postabsorptive young men by injection of a 'flooding dose' of [1-^{13}C]leucine. Clin Sci 77:329-336, 1989

11. McNurlan MA, Essén P, Heys SD, et al: Measurement of protein synthesis in human skeletal muscle: Further investigation of the flooding technique. Clin Sci 81:557-564, 1991

12. McNurlan MA, Essén P, Thorell A, et al: Response of protein synthesis in human skeletal muscle to insulin: An investigation with L-[$^{2}H_5$]phenylalanine. Am J Physiol 267:E102-E108, 1994

13. Park KGM, Heys SD, McNurlan MA, et al: Lymphocyte protein synthesis in vivo: A measure of activation. Clin Sci 86:671-675, 1994

14. Sandgren A, McNurlan MA, Essén P, et al: The effect of stress hormone infusion on protein synthesis rate in skeletal muscle and lymphocytes in healthy volunteers. Clin Nutr 12:28, 1993 (abstr)

15. Bøyum A: Isolation of lymphocytes, granulocytes and macrophages. Scand J Immunol 5:9-15, 1976

16. Doumas BT: Measurement of plasma albumin with bromocresol green. Clin Chim Acta 31:87, 1971

17. Korner A, Debro JR: Solubility of albumin in alcohol after precipitation by trichloroacetic acid: A simplified procedure for the separation of albumin. Nature 178:1067, 1956

18. Gorg A, Posel W, Weser J, et al: Horizontal SDS electrophoreses in ultrathin pore-gradient gels for analysis of urinary proteins. Science Tools 32:5-9, 1985

19. Banauch D, Brümmer W, Ebeling W, et al: Glucose dehydrogenase for the determination of glucose concentrations in body fluids. Klin Chem Klin Biochem 13:101-107, 1975

20. Midgley AR, Rebau RW, Niswender GD: Radioimmunoassays employing double antibody techniques. Acta Endocrinol Suppl 142:247-254, 1969

21. Eisentraut A, Ohneda A, Parada E, et al: Immunological discrimination between pancreatic glucagon and enteric glucagon-like immunoreactivity in tissues and plasma. Diabetes 17:321-322, 1968

22. Dash RJ, England BJ, Midgley AL, et al: A specific, nonchromatographic radioimmunoassay for human plasma cortisol. Steroids 26:647-661, 1975

23. Hallman H, Farnebo LO, Hamberger B, et al: A sensitive method for determination of plasma catecholamines using liquid chromatography with electrochemical detection. Life Sci 23:1049-1052, 1976

24. Calder AG, Anderson SE, Grant I, et al: The determination of low d_5-phenylalanine enrichment (0.002-0.09 atom percent excess), after conversion to phenylethylamine, in relation to protein turnover studies by gas chromatography/mass spectrometry. Rapid Commun Mass Spectrom 6:421-424, 1992

25. Nadler SB, Hidalgo JV, Bloch T: Prediction of blood volume in normal human adults. Surgery 51:224-232, 1962

26. Essén P, McNurlan MA, Wernerman J, et al: Short-term starvation decreases skeletal muscle protein synthesis rate in man. Clin Physiol 12:287-299, 1992

27. Hammarqvist F, von der Decken A, Vinnars E, et al: Stress hormone and amino acid infusion in healthy volunteers: Short-term effects on protein synthesis and amino acid metabolism in skeletal muscle. Metabolism 43:1158-1163, 1994

28. Gore DC, Jahoor F, Wolfe RR, et al: Acute response of human muscle protein to catabolic hormones. Ann Surg 218:679-684, 1993

29. Fryburg DA, Gelfand RA, Jahn LA, et al: Effects of epinephrine on human muscle glucose and protein metabolism. Am J Physiol 268:E55-E59, 1995

30. Louard RJ, Bhushan R, Gelfand RA, et al: Glucocorticoids antagonize insulin's antiproteolytic action on skeletal muscle in humans. J Clin Endocrinol Metab 79:278-284, 1994

31. Gibson JN, Poyser NL, Morrison WL, et al: Muscle protein synthesis in patients with rheumatoid arthritis: Effect of chronic glucocorticoid therapy on prostaglandin F2 alpha availability. Eur J Clin Invest 21:406-412, 1991

32. Preedy VR, Garlick PJ: The effect of glucagon administration on protein synthesis in skeletal muscles, heart and liver in vivo. Biochem J 228:575-581, 1985

33. Essén P, McNurlan MA, Wernerman J, et al: Protein synthesis rate in human skeletal muscle is unaffected by general anesthesia but decreases immediately after abdominal surgery. Am J Physiol 262:E253-E260, 1992

34. Ejesson B, Lind C, Vinnars E, et al: Stress hormones initiate changes in muscle free amino acid concentrations characteristic for surgical trauma. Clin Nutr 9:12, 1990 (abstr)

35. Monjan AA, Collector MI: Stress-induced modulation of the immune response. Science 196:307-308, 1977

36. Pedersen P, Hasselgren PO, Angerås U, et al: Protein synthesis in liver following infusion of the catabolic hormones corticosterone, epinephrine, and glucagon in rats. Metabolism 38:927-932, 1989