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PHARMACOLOGICAL SPARING OF PROTEIN IN BURN INJURY

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The primary goal of the research studies sponsored by this project was to assess the effect of sepsis and/or severe burn injury on the normal anabolic effect of insulin on protein metabolism. All studies were performed in human patients and normal volunteers using stable isotopic tracers and mass spectrometry analysis to quantify various metabolic factors.

The first protocol involved the assessment of the effect of seven days bedrest in normal volunteers on glucose and protein responsiveness to insulin. The results following bedrest served as control values for subsequent studies in patients. The most striking aspect of these studies was the modest effect of bedrest, per se, as compared to the responses observed in bedrested patients following severe burn injury or during sepsis.

Severe burn injury without systemic sepsis induced only a moderate resistance to the action of insulin on glucose uptake and potassium uptake, when compared to the bed-

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rested controls. Sepsis, on the other hand, halved the normal stimulatory effect of insulin on glucose uptake. Potassium uptake, however, remained normally responsive to insulin in septic patients.

The basal rate of protein breakdown was markedly elevated in both septic and burned patients. However, despite the (varying) resistance to the hypoglycemic action of insulin in these patients, the suppressive effect of insulin on protein breakdown remained intact, thereby promoting net protein anabolism. This appeared to be a direct insulin response and not related to a post-receptor stimulation of glucose metabolism, since stimulation of glucose oxidation by means of dichloroacetate, a stimulator of pyruvate dehydrogenase, had no effect on the rate of protein breakdown when insulin remained constant. Furthermore, not only was infused insulin demonstrated to be effective in suppressing protein breakdown, the basal level of insulin was also demonstrated to be effective in both suppressing protein breakdown and curtailing the overall metabolic rate. These latter conclusions were based on experiments in which the basal insulin concentration was reduced by somatostatin infusion.

The general observation that insulin retains its potent protein anabolic effect in severely burned and/or septic patients has obvious clinical implications. However, it is well known that excessive amounts of glucose, given chronically to avoid hypoglycemia during insulin administration, can result in detrimental effects, including excessive fat deposition in the liver and potential ventilatory problems. This might be anticipated to particularly be a problem in burn (not septic) patients, who display only minor resistance to the normal hypoglycemic action of insulin. Consequently, further studies are required to delineate a specific dose-response curve between insulin concentration, suppression of protein breakdown, and fat balance in the liver in order to formulate optimal treatment protocols incorporating the administration of insulin as an anabolic agent.

FOREWORD

For the protection of human subjects the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

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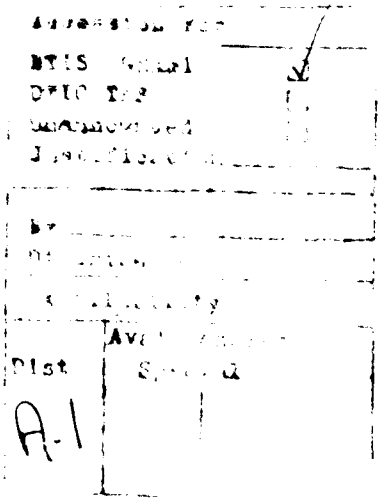

INTRODUCTION:

The primary objectives of the project were as follows:

1). To determine in severely burned patients if lowering the basal insulin concentration by means of the infusion of somatostatin increases the absolute rate of protein breakdown, and the net breakdown of protein, as reflected by urea production.

2). To determine if increasing the concentration of insulin above the basal level (while euglycemia is maintained) inhibits the rate of protein breakdown in severely burned patients.

As described in detail below, these goals have been entirely achieved. The response of burn patients without sepsis has been compared with the response of burn patients with sepsis, as well as with the response of unburned septic patients. The response to stress was distinguished from the effect of embolization by using a group of normal volunteers kept at total bedrest for seven days. The response to bedrest will be discussed briefly, followed by a more indepth description of the response of patients to the manipulation of insulin concentration.

Insulin Responsiveness of Protein Metabolism Following Bedrest:

Insulin regulates both protein and glucose metabolism in vivo. It is established that bedrest itself induces resistance to the hypoglycemic action of glucose (1). We therefore evaluated the effect of seven days of bedrest on the normal action of insulin to suppress protein breakdown. This protocol was necessary in order to distinguish the effects of injury and/or sepsis from the enforced immobilization that accompanies such a major injury.

METHODS:

Subjects:

Six healthy young men, age 21-28 years and 101 ± 3% of ideal body weight (2), participated in the study. Past medical history was negative and none had a family history of metabolic diseases. Physical and screening laboratory examinations were within normal limits. Subjects were admitted to the University of Texas Medical Branch at Galveston Clinical Research Center for a 13-day protocol after providing institutionally approved written informed consent.

Diet during the entire study was a commercial liquid preparation (Ensure, Ross Laboratories, Columbus, OH) at 30 kcal and 1.5 g of protein kg•body wt^{-1}• day^{-1}. During the first 4 days, subjects maintained their usual level of physical activity. Strict confinement to a waterbed commenced on day 6 and continued through day 12. A sequential, five-stage, hyperinsulinemic euglycemic clamp was performed pre-bedrest (day 5) and on the last day of bedrest (day 12). Magnetic resonance imaging (MRI) of the back thigh, and calf muscles was performed both pre-bedrest (day 4) and post-bedrest (day 13); subjects were discharged on day 13. Four of the subjects participated in a third hyperinsulinemic euglycemic clamp performed 2 weeks after this discharge.

Hyperinsulinemic Euglycemic Clamp:

An intravenous catheter was placed in a right forearm vein of overnight-fasted subjects for infusion of the glucose, insulin, and ^{13}C-leucine. A left forearm vein was cannulated for continuous blood glucose monitoring (Biostator, Ames Division of Miles Laboratories, Elkhart, IN). A retrograde catheter was placed in the left dorsal hand vein, and the hand was heated for arterial-

ized blood collection. The bicarbonate pool was primed with 1.0 umol/kg•body weight of $NaH^{13}CO_3$ (99% ^{13}C, Tracer Technologies, Needham, MA). After a prime of 7.2 umol/kg, 1-^{13}C-leucine (99% ^{13}C, Cambridge Isotope Laboratories, Woburn, MA) was infused at 0.12 ± 0.02 umol/kg^{-1}•min^{-1} for 10 h. During the first 2 h, no exogenous insulin was administered. Sequential-primed, 2-h insulin infusions (Humulin Regular, Novo Laboratories, Princeton, NJ) were given at 15, 40, 120 and 240 mU•m^{-2}•min^{-1} to achieve subsequent plasma insulin concentrations of 24 ± 2, 68 ± 2, 239 ± 18, and 718 ± 54, respectively (Figure 1). Whole blood glucose was monitored at 5- to 10-min intervals (YSI Glucose Analyzer, Yellow Springs Instruments, Yellow Springs, OH) and maintained at 85 ± 3 mg/dl by variable infusion of aqueous dextrose (15 g/dl) as shown in Figure 1. Glucose infusion was tapered as appropriate for euglycemia after termination of insulin administration. Circulating potassium was monitored hourly during the procedure and maintained at 4.3 ± 0.1 meq/l by infusion of KCl 50 meq/l at 30, 60, 90, 120, and 150 ml/h during the five study periods. Subjects remained awake and supine throughout the clamp study.

Arterialized venous blood was withdrawn and expired air collected into 3-liter anesthesia bags at 10-min intervals over the final 30 min of each 2-h infusion period. Total blood volume withdrawn was ~280 ml for the 10-h duration. Heparinized blood was immediately centrifuged, and plasma stored at -20 degree C until analyzed for ^{13}C enrichment of leucine and alpha-ketoisocaproic acid (KICA) and for amino acid concentrations. Carbon dioxide from expired breath was immediately trapped at $NaHCO_3$ by bubbling through 10 ml of 0.1 N NaOH. At each insulin infusion rate, measurements of CO_2 production ($\dot{V}CO_2$),

O_2 consumption ($\dot{V}O_2$), and respiratory quotient (RQ) were performed using a metabolic cart (Beckman Instruments, Fullerton, CA).

Four additional studies employing infusions of 5,5, 5-d_3-leucine in addition to the 1-^{13}C-leucine were performed to quantitate the potential impact of label recycling in our 10-h studies. After a priming dose of 2.4 umol/kg of 5,5, 5-d_3-leucine was given, infusions of 0.04 umol/$kg^{-1} \cdot min^{-1}$ were administered for 2 h either in the baseline period (2 subjects) or in the fifth period (2 different subjects) of the 10-h full euglycemic clamp. Blood samples were obtained, processed, and analyzed as above and as follows. If significant recycling of the ^{13}C label occurred, the Ra of leucine in the fifth period calculated from ^{13}C isotopic enrichment data (10-h infusion) would be less than that calculated from the deuterium data (2-h infusion).

Analytical Procedures:

Plasma amino acid levels were measured by ion exchange chromatography using an autoanalyzer (Model 121M, Beckman Instruments). Leucine and KIC enrichment were measured as previously described using a Hewlett-Packard 5985 gas-chromatograph mass-spectrometer (3). $^{13}CO_2$ enrichment was determined using an isotope-ratio mass-spectrometer (3), and overall CO_2 production (CO) and oxygen consumption ($\dot{V}O_2$) were measured by indirect calorimetry. Urinary nitrogen excretion was determined on 24-h collections, and urinary excretion of 3-methyl-L-histidine was determined on each sample with an amino acid analyzer (Model 6300, Beckman Instruments). Urine content of free cortisol (4) and catecholamine metabolites metanephrine and nor-metanephrine (5) were determined pre-bedrest (days 3 and 4) and during bedrest (days 10 and 11).

Creatinine content of each urine sample was measured by autoanalyzer (Beckman Instruments).

On days 4 (control) and 11 (bedrest), blood was drawn at 0800 from overnight fasted subjects for radioimmunoassay of circulating insulin (6), cortisol (4), glucagon (7), growth hormone (8), and somatomedin C (9). Subsequently, a 3-h oral glucose tolerance test was performed (2).

MRI of the back, thigh, and calf muscle volume was made both pre-bedrest (day 4) and post-bedrest (day 13), using a Technicare MRI imager equipped with a 0.6-T superconducting magnet (Technicare, Solon, OH) (10).

Calculations:

Ra of plasma leucine and intracellular leucine were calculated as follows:

$$Ra = \frac{F}{IE} - F'$$

where F is the rate of ^{13}C-leucine tracer infusion and F' is the total infusion rate of leucine, in $umol/kg^{-1} \cdot min^{-1}$. In the case of Ra of plasma leucine, IE is the isotopic enrichment of plasma leucine; to determine Ra of intracellular leucine, IE is the isotopic enrichment of KICA (IE_{KICA}), its keto acid analogue. Isotopic enrichment is expressed in atom percent excess. Ra intracellular leucine should be a direct reflection of the absolute rate of protein breakdown, provided that the average composition and rate of breakdown of the proteins whose breakdown contribute most prominently to the plasma flux does not differ from that of the whole-body protein. Leucine oxidation rate was calculated by the following equation (3):

$$\text{Oxidation} = \frac{^{13}CO_2 \text{ excretion } (umol\ kg^{-1} \cdot min^{-1})/0.8}{IE_{KICA}}$$

These same calculations were used in all aspects of this project.

Because these studies involved infusion of large quantities of glucose known to be enriched with ^{13}C (11), a multi-dose sequential euglycemic clamp was repeated on four of the subjects without ^{13}C-leucine tracer infusion. Figure 2 shows the $^{13}CO_2$ enrichment of expired air in these studies, as a function of glucose infusion rate. It can be seen that during hyperinsulinemia, metabolism of ostensibly unenriched glucose increased the expired air $^{13}CO_2$ enrichment, especially at higher rates of insulin and glucose infusion. Therefore, the measured $^{13}CO_2$ enrichments of our studies using ^{13}C-leucine tracer were adjusted for the effect of glucose infusion rate on $^{13}CO_2$ excretion.

Data are expressed as means $\pm$ SE. For data taken during a steady-state plateau, i.e., each step of the euglycemic clamp, an average value was taken over the last 30 min of the plateau. Differences between means were judged statistically significant at the 95% confidence level, using analysis of variance (ANOVA) repeated measures and Scheffe's post-hoc test. Most statistical calculations were performed on a microcomputer using ABstat version 5.02 software (Anderson-Bell, Parker, CO).

Results:

Glucose Tolerance and Hormone Levels:

Before bedrest, subjects were found to have normal glucose tolerance, as judged by a 3-h 75-g oral glucose tolerance test (Table 1). After 6 days of bedrest, the glucose tolerance test revealed mildly decreased glucose tolerance with a 16% increase in area under the glucose curve ($p < 0.05$). Despite basal glucose levels being equal before and during bedrest, basal insulin was

elevated 43% ($p < 0.05$) after 6 days of bedrest. Oral glucose challenge prompted an exaggerated insulin secretory response, with a 44% increase in the area under the insulin curve ($p < 0.05$). Circulating levels of growth hormone and somatomedin C were not altered from control at 6 days of bedrest. In contrast, plasma glucagon was elevated 17% ($p < 0.05$) in bedrested subjects. There was a trend toward increased plasma cortisol that failed to reach statistical significance, but the 24-h urinary free cortisol excretion revealed a 16% increase ($p < 0.05$) after bedrest. Urinary excretion of metanephrine (an indication of epinephrine secretion) was unchanged and nor-metanephrine (metabolite of norepinephrine) was decreased by 15% ($p < 0.05$) by bedrest.

Magnetic Resonance Imaging of Back and Lower Extremities:

The bilateral thigh and calf soft tissue compartment volumes are shown in Table 2. It can be seen that the calf and thigh muscle compartments decreased by 1.0 and 1.6%, respectively, after 7 days of bedrest. In contrast, the concomitant increases in calf and thigh fat volumes (1.9 and 5.0%) were not statistically significant ($p < 0.09$). The paraspinal muscle volume atrophied by 4.2% ($p = 0.02$) during the bedrest period.

Nitrogen Balance and 3-Methyl-L-Histidine Excretion:

During the first 4 days of the study, when subjects maintained ad libitum activity and established a nitrogen equilibrium on the liquid diet, the subjects exhibited a positive nitrogen balance (Table 3). Not shown in Table 3 are the negative nitrogen balances of 2.7 ± 0.5 g exhibited by all subjects on the days of the euglycemic clamps (days 5 and 7), when about half of the calories were supplied by intravenous glucose, and consequently protein intake was reduced to 0.4 g $kg^{-1} \cdot day^{-1}$ to keep caloric intake constant at 30 kcal

$kg^{-1} \cdot day^{-1}$. During bedrest (days 6 through 11), when the pre-bedrest diet was continued, nitrogen balance was less positive than that before bedrest. It has been shown that when N intake in humans exceeds the predicted need of 0.5 $g \cdot kg^{-1} \cdot day^{-1}$, there is an apparent positive N balance that does not represent net protein deposition. Therefore, the N-balance effects of bedrest were also calculated as the net difference compared with the baseline period. This revealed a negative nitrogen balance beginning on the second day of enforced bedrest and extending over the 6 days before repeat of the euglycemic clamp, with an average cumulative loss of 6.3 g of nitrogen by the sixth day. Urinary excretion of 3-methyl-L-histidine data are also shown in Table 3. Excretion of 3-methyl-L-histidine increased slightly (11%) during bedrest but statistical significance was not achieved (mean baseline 273 $\pm$ 15 versus mean bedrest 304 $\pm$ 12, $p = 0.06$). Because creatinine excretion also increased slightly, when 3-methyl-L-histidine excretion was expressed per micromoles of creatinine excretion, there was no difference in 3-methyl-L-histidine excretion between the control period and the bedrest period.

Multi-Dose Sequential Euglycemic Clamp:

Insulin infusion at 0, 15, 40, 120, and 240 $mU \cdot m^{-2} \cdot min^{-1}$ resulted in a plasma insulin level of 7 $\pm$ 1, 24 $\pm$ 2, 68 $\pm$ 2, 239 $\pm$ 18, and 718 $\pm$ 54 uU/ml, respectively, in control subjects. After 7 days of bedrest, the same infusion rates produced circulating levels of 9 $\pm$ 1, 25 $\pm$ 1, 66 $\pm$ 1, 220 $\pm$ 7, and 736 $\pm$ 31 uU/ml, respectively (not significant). Figure 2 displays the euglycemic clamp data for our six subjects in the baseline period. After a 2-h control period, four 2-h stepwise increasing insulin infusions were given. The solid vertical bars represent the glucose infusion rates necessary to maintain

euglycemia in the last 30 min of each 2-h infusion period. Figure 1 shows that the glucose infusion rate was relatively stable in the last 30 min of each study period. The blood glucose was maintained very close to 85 mg/dl throughout the insulin infusion periods (Figure 1). Plasma isotopic enrichment of leucine and KICA were also very stable in the last 30 min of each study period as shown in Figure 3. The ratio of KICA enrichment to leucine enrichment was 0.60 during the control period (study period 1) and increased slightly to 0.75 in the period of maximal glucose infusion (period 5). The ^{13}C enrichment of expired CO_2 from ^{13}C-leucine was stable during the last 30 min of each 2-h study period as shown in Figure 4. The data shown in Figures 1, 3, and 4 suggest that in the final 30 min of each 2-h study period of the sequential multi-dose euglycemic clamp procedure, reasonable stability occurs for insulin modulation of both glucose and leucine metabolism.

Table 4 contains the individual subject data for plasma leucine ^{13}C-enrichment for each euglycemic clamp study period for both pre- and post-bedrest clamp procedures. Table 5 shows similar data of KICA enrichment from infused 1-^{13}C-leucine. The data from Table 5 were used to calculate the Ra intracellular leucine as described in Methods. Figure 5 shows graphically the effect of exogenous insulin on the Ra of intracellular leucine. This use of reciprocal pool enrichment provides a better assessment of intracellular leucine dynamics than the enrichment of the plasma leucine (12, 13), since intracellular leucine and plasma KICA are in equilibrium. Use of plasma ^{13}C-leucine enrichment from infused ^{13}C-leucine results in a systematic underestimate of the leucine release from intracellular protein breakdown. Basal Ra intracellular leucine before and during bedrest were not different. Insu-

lin caused a dose-dependent reduction of Ra intracellular leucine in both active and bedrested subjects with maximal effect (41% inhibition, $p < 0.001$) achieved at an insulin concentration of 226 uU/ml. Half-maximal response required 38 before and 36 uU/ml after bedrest. At no insulin concentration did the Ra of intracellular leucine differ between the pre-bedrest and bedrest periods.

Recycling of infused labeled leucine has been demonstrated by Schwenk and co-workers with infusion duration as long as 24 h was used (14). To determine the impact of incorporation and subsequent release of 1-^{13}C-leucine on calculated leucine Ra, we performed doubly labeled leucine infusions of differing durations as described in Methods. Table 6 shows the results of these studies. Even though the study design is complex (Experiments 1 and 2 were baseline studies and 3 and 4 were done during maximal insulin infusions), there is no evidence of recycled label causing significant erroneous quantitation of the Ra leucine.

Leucine oxidation is calculated from the ^{13}C-enrichment of expired CO_2, the $\dot{V}CO_2$ rate from the indirect calorimetry, and the plasma KICA enrichment. Figure 4 shows the CO_2 enrichment data and Figure 6 displays graphically the effect of insulin on $\dot{V}CO_2$. As is shown, $\dot{V}CO_2$ increases with increasing insulin doses (and consequent increased glucose utilization) and the insulin stimulation of $\dot{V}CO_2$ was not altered significantly by bedrest. Leucine oxidation at various insulin concentrations calculated from these data is shown in Figure 7. It can be seen that insulin causes a dose-dependent inhibition of leucine oxidation before and after bedrest. There was a trend toward decreased leucine oxidation during bedrest at each insulin dose, but no value

was statistically different from control. Maximal inhibition of leucine oxidation (54%, $P < 0.001$) was achieved at an insulin concentration of ~226 uU/ml.

Whole-body protein catabolism can be estimated as the rate of appearance of intracellular leucine, divided by 590 umol leucine/g protein. Whole-body protein catabolism in our bedrested subjects was not different from control at any insulin concentration (Table 7). Insulin inhibited proteolysis in both active and bedrested subjects, in a dose-dependent manner, with a 41% maximal suppression ($p < 0.001$). Because calculated protein breakdown does not differ between control and bedrested subjects at any insulin infusion, these data indicate that (in the absence of protein intake) protein breakdown was not accelerated during bedrest and furthermore that insulin-mediated suppression of proteolysis was unimpaired by bedrest.

Plasma amino acid levels are shown in Table 8. Insulin caused a dose-dependent reduction in most circulating amino acids both before and during bedrest. Those amino acids that showed the largest effect were isoleucine (decreased 86%), methionine (decreased 73%), tyrosine (decreased 61%), valine (decreased 60%), and leucine (decreased 59%). Aspartate and histidine levels were not significantly reduced by insulin before or after bedrest. In addition, the effect of insulin on circulating glycine and alanine was minor in all subjects (23-29%). When bedrested subjects were compared with their pre-bedrest control, there was no difference in plasma amino acid levels at any insulin concentration.

Discussion:

Seven days of bedrest in the present study was sufficient to cause a

significant decrease in the sensitivity of glucose metabolism to insulin. This is consistent with reports in the literature involving from 3 to 14 days of physical inactivity (14, 15). Concurrently, MRI analysis of the back and lower extremities revealed that muscles of the back and lower extremity atrophied during the seven-day bedrest. The changes were particularly marked in the paraspinal region, where the continued supine position reduced their work despite the fact that the lower extremities remained free to move. The smaller changes in the thigh and calf may be due to continued movement during the bedrest. Muscle atrophy and increased fat deposition in the thigh during inactivity is in agreement with a previous report using serial CT scans (16). Kristensen, et al (16) found a 21% decrease in thigh muscle cross-sectional area coupled with a 22% increase in fat cross-sectional area after 6-8 week of immobilization. If one assumes that these changes occurred at a constant rate, this would correspond to ~3-~4% decrease or increase, respectively, per week. That fat is being deposited while muscle volume is decreasing could help explain why the immobilized subjects do not lose body weight, despite the presence of a protein catabolic state (17).

Subjects in our study had a consistently negative nitrogen balance during the bedrest period as compared with the control period. The decreased net nitrogen balance during bedrest in the present study is consistent with the findings of earlier studies (17, 18). However, Dietrick, et al (17) and Schoenheyder, et al (18) reported an absolute negative N balance in their inactive subjects that in the present study was only apparent on the two days of the euglycemic clamps, when protein intake was acutely reduced to ~0.4 g/kg, day. One possible explanation for the discrepancy is the degree of inacti-

vity. Using plaster casts, Dietrick, et al (17) completely immobilized their subjects and Schoenheyder, et al (18) immobilized the lower body. In contrast, our subjects, while restricted to a supine position in bed, were not rigidly immobilized and were free to roll in bed.

Another theoretical consideration is the protein intake, since provision of amino acids augments protein synthesis in vivo in humans (19). However, the 1.5 g protein $kg \cdot body \cdot wt^{-1} \cdot day^{-1}$ used in the present study is roughly comparable to the 1.4 g protein $kg \cdot body \cdot wt^{-1} \cdot day^{-1}$ provided by Dietrick, et al (17) and the ~1.25 $g \cdot protein \cdot kg^{-1} \cdot day^{-1}$ given by Schoenheyder, et al (18). It is interesting that the onset of relative negative nitrogen balance occurred 5 days after commencement of immobilization in the earlier studies (17, 18), but started from day 2 of bedrest in our studies. This earlier net negative nitrogen balance may be in part due to the drop in protein intake due to the euglycemic clamp study on the day before the bedrest began in the present study.

Seven days of bedrest did not cause changes in plasma amino acid profile. This is in agreement with findings of Askanazi, et al (20) on subjects after 4 days of bedrest. The present study further revealed that the depression of plasma amino acids by hyperinsulinemia is not impaired by strict bedrest. It should be emphasized that our subjects were adequately nourished both in terms of nitrogen and caloric requirements and that subjects on a hypocaloric protein-free diet during bedrest may exhibit elevated plasma branched-chain amino acids (20).

Myofibrillar breakdown releases 3-methyl-L-histidine from protein and this amino acid cannot be reincorporated (21). In humans, skeletal muscle

contains by far the largest pool of 3-methyl-L-histidine, accounting for over 90% (21). Although skin and the gastrointestinal tract also produce 3-methyl-L-histidine, and the turnover rate of those tissues is rapid relative to muscle, a maximum of 25% of 3-methyl-L-histidine excretion can be attributed to non-muscle sources (21). Therefore, because our subjects were given a diet free of animal protein, their urinary excretion of 3-methyl-L-histidine should have been a reflection of the rate of their endogenous muscle protein breakdown. The failure of bedrest to increase 3-methyl-L-histidine release significantly indicates that muscle protein breakdown was not increased by inactivity. Furthermore, the isotope studies showed that there was no difference in whole-body protein breakdown consequent to bedrest, as reflected by the rate of leucine release. Whole-body protein breakdown was inhibited by insulin in a dose-dependent manner, a finding consistent with earlier studies (5, 19). Our data show further that the insulin responsiveness of protein breakdown in active humans is unchanged following 1 week of bedrest.

The pioneering work by Schoenheyder, et al (18) suggests that the defect in protein balance during bedrest is decreased by protein synthesis. More recently, Gibson, et al (22) have looked at quadriceps muscle protein synthesis in active and inactive (casted) limbs. They found that protein synthesis was decreased 26% in the quadriceps immobilized for 5 weeks compared with the contralateral non-immobilized muscle. It is clear from the MRI analysis of skeletal muscles in the present study that the inactive muscles significantly decreased in volume over the 7 days of bedrest. Because this occurred at a time when protein breakdown (assessed by either whole-body leucine kinetics or 3-methyl-L-histidine excretion) was apparently not increased, the most likely

explanation for the skeletal muscle atrophy in the present study is decreased in protein synthesis.

It is possible to generate an estimate of whole-body protein synthesis using the rate of appearance of intracellular leucine and "plasma" leucine oxidation (3). By this method, whole-body protein synthesis before and during bedrest was 221 $\pm$ 12 and 216 $\pm$ 10 mg$\cdot$protein$\cdot$kg$^{-1}\cdot$h^{-1}, respectively (not significant). However, this result is an indirectly derived value that requires a number of assumptions that are either untested or known to be untrue. For example, it is assumed that leucine is distributed homogenously in the body's protein pool. Even if all assumptions implicit in the calculation of synthesis are true, the results are of questionable value in interpreting the mechanism for muscle atrophy in bedrest, since the euglycemic clamp data are generated on fasted subjects (particularly with regard to protein intake), whereas during much of the time, the bedrested subjects were in the fed state. Protein synthesis, in contrast to protein breakdown, is a process exquisitely sensitive to the nutritional status of the subject (23). Muscle protein synthesis, in particular, increases as much as 100% during the fed state (3). Furthermore, it has recently been demonstrated that indomethacin can block the feeding-induced stimulation of protein synthesis without altering protein synthesis in the fasted state (23). Therefore, it is more appropriate to evaluate skeletal muscle protein synthesis directly and during the fed state which represents the time when one could expect differences to be manifested.

Leucine oxidation in both active and inactive subjects was markedly inhibited by hyperinsulinemia. A comparable dose-dependent reduction of leucine

oxidation by insulin has also been reported by others (19), who utilized ^{14}C-labeled leucine as tracer. Fukagawa, et al (22), however, did not find a diminished leucine oxidation during the hyperinsulinemic euglycemic clamp. The failure of Fukagawa, et al (22) to detect the suppression of leucine oxidation by insulin is likely due to the $^{13}CO_2$ production from infused glucose during the hyperinsulinemic period. We found that $^{13}CO_2$ enrichment of expired breath is augmented during the euglycemic clamp, even with no ^{13}C-leucine tracer infusion, in a manner directly related to the glucose infusion rate (Figure 2) because the glucose is naturally enriched with ^{13}C-glucose as compared with fat. The hyperinsulinemic euglycemic clamp changes the energy metabolism of subjects, as evidenced by an insulin dose-dependent increase in RQ from 0.84 $\pm$ 0.01 basal to a maximum of 1.01 $\pm$ 0.01 in both active and inactive subjects ($p < 0.001$). Therefore the primary fuel source in the basal state, lipid, is supplanted by carbohydrate in the presence of exogenous insulin. Increased glucose oxidation, relative to fat oxidation, would yield additional $^{13}CO_2$ in expired breath from both exogenous glucose and endogenous carbohydrate stores. If a correction is not made for the $^{13}CO_2$ production caused by augmented glucose metabolism during the hyperinsulinemic clamp, then a falsely elevated $^{13}CO_2$ production from ^{13}C-leucine would lead to erroneously high estimations in leucine oxidation. Our data demonstrate (Figures 2 and 4) that as much as 50% of the $^{13}CO_2$ expired at the highest insulin infusion is derived from sources, exogenous and endogenous, other than the infused 1-^{13}C-leucine. Therefore, we believe that our data more accurately represent leucine oxidation during euglycemic hyperinsulinemia than the data of Fukagawa, et al (22). Natural enrichment of glucose with ^{14}C is negligibly small,

however, and therefore the studies by Tessari, et al (24) and Umpleby, et al (25) are not subject to falsely high $^{14}CO_2$ enrichment from glucose.

In conclusion, these data show that 7 days of bedrest caused measurable atrophy of inactive skeletal muscle and a net increase in urinary nitrogen loss. However, protein degradation was unchanged as indicated by the intracellular rate of appearance of leucine and no change in urinary 3-methyl-L-histidine excretion. Despite evidence of bedrest-induced resistance to insulin's action on glucose metabolism, the sensitivity of protein metabolism in general, and leucine metabolism in particular, to insulin remained intact. We therefore deduce that the protein wasting secondary to bedrest appears to be primarily the result of reduced protein synthesis. As will be seen when evaluating subsequent sections of this report in which the response of patients is evaluated, perhaps the most striking aspect of the response of normal volunteers in bedrest is the relatively trivial nature of the increased protein breakdown.

DIFFERENTIATION BETWEEN SEPTIC AND POST-BURN INSULIN RESISTANCE

At the outset of this project, it was unclear if the nature of the "insulin resistance" in septic patients is identical to that following burn injury. Furthermore, the relationship between alterations in insulin's effectiveness to stimulate glucose uptake in these pathological circumstances and its effect on potassium uptake is not clear. Although insulin's action on glucose metabolism has been linked historically to that on potassium uptake (26, 27), a growing body of evidence indicates that the coupling of these two responses may not necessarily be as close as previously thought (28-30). Andres, et al (28) showed that insulin infusion in the perfused forearm had both hypoglycemic and hypokalemic effects, but the time course of the potassium uptake was more rapid than that of glucose, indicating that transport of potassium into cells did not depend on glucose entry. Experiments on incubated skeletal muscle have shown that insulin can induce potassium uptake even in the absence of glucose, and that blockade of potassium uptake does not interfere with insulin-stimulated glucose uptake (31, 32). In addition, the liver plays an important role in whole-body clearance of potassium in response to insulin, and this clearance occurs independently of glucose flux across the splanchnic bed (29). Finally, it has been reported recently in patients with hypertension or type II diabetes mellitus that the resistance to the hypoglycemic action of insulin is not matched by a simultaneous impairment of potassium uptake (30).

Therefore, the purpose of this study was to determine the maximal biological effectiveness of exogenously administered insulin on glucose uptake in septic and burned patients, and to examine the relationship between the

glucose- and potassium-uptake responses to the infused insulin. The same data was collected in the volunteers described in the previous section who were put to bedrest for 7 days, and these subjects served as controls.

MATERIALS AND METHODS:

Subjects:

Eight septic patients and eight non-septic patients recovering from severe burn injury were studied in the Medical, Surgical and Burn Intensive Care Units at John Sealy Hospital after giving written informed consent approved by the Institutional Review Board of the University of Texas Medical Branch. Sepsis was defined as documented infection in the cerebrospinal fluid, blood, lung, peritoneum, urinary tract, and in wounds or abscesses, coupled with fever, leukocytosis, thrombocytopenia, altered mental status, and hyperdynamic cardiovascular status. The average age of the septic patients was 38 $\pm$ 2 (SE) years, with a range of 28 to 44 years (Table 9). At the time of the study, patients had an average sepsis score (33) of 17 $\pm$ 1, with a range from 12 to 22. By the APACHE II classification (34), the average score was 15 $\pm$ 1, with a range from 8 to 25. Septic patients had a mean cardiac index of 6.33 $\pm$ 0.41 $L/min^2 \cdot min$ and an average systemic vascular resistance index of 809 $\pm$ 48 $dynes/m^2 \cdot mmHg$. Septic patients were studied by the hyperinsulinemic euglycemic clamp technique (35) within 48 hours of the diagnosis of sepsis. The non-septic burn patients had an average burn size of 63% $\pm$ 5% total body surface with a range of 45% to 83% (Table 2). Full-thickness area (third degree burn) was 35% $\pm$ 10% total body surface, with a range of 4% to 80%. They had an average injury severity score (36) of 32 $\pm$ 2 with a range

from 25 to 41. These burn patients were studied during the hypermetabolic "flow" phase, at postburn day 11 ± 1.

Euglycemic Clamp:

An eight-hour metabolic study was begun at 6:30 a.m. on the post-absorptive patients. Insulin, potassium, glucose, and isotopes were infused through a central venous catheter. Blood samples were withdrawn via an arterial catheter. In four septic and three burn patients, a primed-constant infusion of 6, 6-d_2-glucose (99% ^2H, MSD Isotopes, Montreal, Canada) was administered at 0.055 ± 0.003 mg/kg•min with a priming dose of 4.4 mg/kg. During the first 3 hours, the basal period, no exogenous insulin or glucose was infused. Subsequently, a primed-constant infusion of insulin (Novolin Regular, Novo Laboratories, Princeton, NJ) was administered for 5 hours. The infusion rates for the volunteers, patients with sepsis, and severely burned patients were 240, 400 and 500 mU/M^2, respectively. The different infusion rates were used because of reports of increased insulin clearance in burned patients (37, 38). Plasma glucose, monitored at 5-minute intervals during hyperinsulinemia (Autoanalyzer, Beckman Instruments, Fullerton, CA), was maintained at 90 ± 3 mg/dL by variable infusion of aqueous glucose (20 g/dL). Serum potassium was monitored at 30 to 60 min intervals (Autocal IL 643 Flame Photometer, Instrumentation Laboratory, Lexington, MA) and maintained within normal limits by variable infusion of KCl (20 mEq/dL in 0.45 NaCl). During the final 1 hour of the study, serum potassium was clamped at 3.9 ± 0.1 mEq/L.

Arterial blood samples were taken at 15-minute intervals during the final hour of the basal period or the final 90 minutes of the hyperinsulinemic period for analysis of isotopic enrichment of the glucose. Total blood volume

withdrawn was 160 mL including the aliquots for plasma glucose and potassium concentrations. Blood was kept on ice until the end of the study, immediately centrifuged, and the plasma stored at -20 degree C until analyzed. Indirect calorimetry (Horizon, Beckman Instruments) was performed during both the basal and hyperinsulinemic periods to measure CO_2 production ($\dot{V}CO_2$), O_2 consumption ($\dot{V}O_2$), respiratory quotient (RQ; VCO_2[mL/min]/VO_2[mL/min]), and resting energy expenditure (REE). The non-protein RQ, which corrected for nitrogen excretion, was used for the calculation of glucose oxidation (39).

Analytical procedures were as described in the previous section.

RESULTS:

Baseline Data:

Septic patients tended to be somewhat older than the volunteers ($p < 0.02$), but there was no significant difference in body weight between any of the three groups (Table 1). However, both body mass index and percent ideal body weight were significantly higher in the patients with sepsis than in the other two groups. Septic and burned groups both had RQ values that decreased below 0.8. Their metabolic rates were about 50% higher than those predicted by the Harris-Benedict equation and 67% above those of bedrested volunteers ($p < 0.001$). Compared with bedrested control values, fasting plasma glucose of both groups also increased almost 40% ($p < 0.02$) despite a comparable circulating insulin level. There was no difference in any of these parameters between septic patients and those with severe burn injury uncomplicated by sepsis. However, basal endogenous glucose production in the septic patients decreased by 24% compared with the burned patients ($p < 0.01$). This decrease indicates that, because the plasma glucose concentrations were identical

between the two groups, the basal glucose clearance in the septic patients also decreased compared with that of burn patients despite a comparable circulating insulin level.

Euglycemic Clamp:

A primed constant infusion of insulin at 240 $mU/m^2/min$, 400 $mU/m^2/min$, and 500 $mU/m^2/min$ in bedrested volunteers, septic patients, and burned patients, respectively, yielded corresponding plasma insulin concentrations of 736 $\pm$ 31, 1298 $\pm$ 247 and 1213 $\pm$ 229 mU/mL (NS). Hyperinsulinemia prompted a brisk cellular uptake of K^+ from the vascular compartment in both groups of patients, and the rates of infusion of K^+ necessary to maintain constant plasma K^+ levels were not significantly different (Figure 8). Plasma K^+ decreased significantly ($p < 0.05$) from the basal level within the first 15 minutes of hyperinsulinemia despite an increasing K^+ infusion rate above basal maintenance, as shown in Figure 8. Initial repletion of plasma K^+ was intentionally conservative to avoid hyperkalemia, particularly in the septic patients in whom renal K^+ handling might have been compromised. However, after 4 hours of hyperinsulinemia, a steady state was achieved in which the K^+ infusion rate equaled that of K^+ disposal from the vascular space, with a stable plasma K^+ concentration in all subjects of 3.9 $\pm$ 0.1 mEq/L. The steady state K^+ clearance (K^+ infusion rate divided by serum concentration) of septic and burned patients is compared with that of bedrested volunteers in the left side of Figure 9. Maximal potassium clearance during bedrest was 0.54 $\pm$ 0.06 mL/kg•min. Septic patients had a K^+ clearance of 1.53 $\pm$ 0.21 mL/kg min, while that in burn patients was intermediate at 1.08 $\pm$ 0.12 mL/kg•min ($p < 0.05$ ver-

sus controls). There was no difference in K^+ clearance between septic and burned patients.

Insulin also stimulated whole-body glucose uptake by the septic and burned patients (Figure 10). Burned patients showed a relatively rapid and large glucose uptake in response to insulin, which reached a steady state by approximately 2 hours. In contrast, septic patients reached a steady state more slowly (after about 3 hours) and required less exogenous glucose to maintain the same plasma glucose concentration. Both of the patient groups reached a steady-state glucose uptake more slowly than did volunteers, who reached a steady state in 30 to 60 minutes. The steady-state glucose clearance (glucose infusion rate divided by plasma concentration) of septic and burned patients is compared with that of bedrested volunteers in the right side of Figure 10. It can be seen that maximal insulin-stimulated glucose clearance of the septic patients decreased by 48% and 43% ($p < 0.001$), compared with controls and severely burned patients, respectively. However, there was no difference in clearance between bedrested controls and burned patients. When glucose uptake of bedrested volunteers, septic patients, and burned patients was determined at a lower insulin concentration, with insulin infused at 120 $mU/m^2/min$ in volunteers and at 240 $mU/m^2/min$ in the patients, the plasma insulin levels of the three subject groups were again comparable (Table 12). As was the case during the higher insulin infusion, glucose uptake in septic patients decreased by 49% compared with either bedrested volunteers or non-septic burned patients ($p < 0.05$). There was also no difference between volunteers and non-septic burn patients at this insulin dose.

These data show that in septic patients, exogenous glucose uptake in response to insulin is impaired. To test whether this impairment is caused by a peripheral defect or a failure of insulin to suppress hepatic gluconeogenesis in septic patients, 6, 6-d_2-glucose was infused in four septic patients and three burn patients, with all aspects of the experimental protocol as described above. Endogenous glucose production in both septic and burned patients was completely suppressed to -0.02 $\pm$ 0.29 and +0.08 $\pm$ 0.78 mg/kg•min, respectively, during insulin infusions. Therefore, it seems that in septic patients the reduced ability of insulin to promote exogenous whole-body glucose utilization is not caused by competition from endogenously produced glucose.

The indirect calorimetry data are shown in Table 13. ($\dot{V}O_2$ and $\dot{V}CO_2$ were significantly stimulated by the glucose clamp procedure in the burn patients, but not in septic patients. In both groups, the rate of glucose oxidation increased during clamping. The magnitude of increase was larger in the burn patients, but the difference was not significant. Furthermore, during clamping the percent of glucose uptake that was oxidized increased significantly in the septic but not the burned patients (Table 13). The percent energy derived from glucose oxidation during clamping was not different in the two groups (64.6% $\pm$ 8.6% versus 53.2% $\pm$ 3.3%, respectively).

Figure 10 shows potassium clearance and glucose clearance in the bedrested volunteers, septic patients, and burn patients. The individual data are shown to amplify the point made earlier about the mean data for the same factors (Figure 2); the septic patients had the largest potassium clearance yet the lowest rate of glucose clearance of the three subject groups. For each

patient group, there was no significant relationship between insulin-induced potassium and glucose clearance.

DISCUSSION:

This study was designed to elucidate if the insulin responsiveness of potassium and glucose metabolism are similar in patients with sepsis and severely traumatized burn patients who are not septic. The results show that insulin-stimulated potassium uptake in both patient groups was equal to or greater than that in the control subjects. In contrast, insulin-mediated maximum glucose uptake in septic patients was markedly impaired, whereas that of burn patients was comparable to that in bedrested controls. In both groups of patients, insulin-mediated stimulation of potassium and glucose uptake was dissociated. Although a post-receptor defect in glucose metabolism in septic patients could explain these results, the indirect calorimetry data do not support an impairment in the ability to oxidize glucose.

A depletion of total-body potassium stores in both the septic and burned patients, despite maintenance of normal plasma K^+ concentration, may have contributed to the augmented potassium clearance in response to insulin (40). Lehr, et al (41) found that total-body potassium was depleted by 30% to 40% in critically-ill surgical patients. A decrease in intracellular K^+ would make the concentration gradient more favorable for cellular uptake of potassium. There are several mechanisms to account for wasting of potassium stores. First, the Na-K adenosine triphophatase (ATPase) system is inhibited in sepsis (42). Second, microvascular acidosis can promote cellular uptake of H^+ in exchange for intracellular K^+ (43). Third, increased plasma levels of corti-

sol, glucagon and epinephrine can interfere with net cellular uptake of potassium and exacerbate losses through the kidneys and gastrointestinal system (44-47). Last, many of the septic and burned patients had earlier been treated with furosemide for diuresis, which would cause an iatrogenic urinary potassium loss. Therefore, even though several factors may contribute to the increased K^+ clearance by the patients compared with volunteers, it is clear that insulin-stimulated K^+ clearance is not inhibited in these patients. Furthermore, the time course of potassium uptake was much more rapid than that of glucose (15 minutes versus 120 minutes to reach steady-state), indicating that potassium uptake did not require a concomitant glucose uptake in either septic or burned patients. These findings are in agreement with those of Andres, et al (28) in normal volunteers.

The difference in glucose uptake responses to insulin shows that the so-called "insulin resistance" of sepsis and burn injury cannot be considered synonymous, unless the burned patient becomes septic. The three burned patients who became septic did not have more severe burn injuries than their non-septic counterparts (64% $\pm$ 11% versus 63% $\pm$ 5% total body surface for the septic and non-septic burn patients, respectively). However, maximal glucose infusion in these three septic patients was 7.53 $\pm$ 0.76 mg/kg•min, which is similar to that of septic patients with other primary insults (6.06 $\pm$ 0.52 mg/kg•min), and quite different from the average value of 12.4 $\pm$ 0.72 mg/kg•min in non-septic burn patients. Although it is true that the septic patients were slightly obese (128% ideal body weight), such a small magnitude of obesity could not have been the cause of such large differences in glucose clearance between the septic patients and the other groups. Differences in glucose

infusion rates needed to maintain euglycemia can be equated to glucose uptake because endogenous glucose production in both septic and burn patients was completely suppressed during the hyperinsulinemic clamp.

The impaired peripheral glucose uptake of septic patients is consistent with the findings in previous studies at a lower insulin level of 50 uU/ml (48). However, the results of the present study show that, in contrast to normal volunteers, who reach a steady-state glucose uptake in 30 to 60 minutes (49), 3 hours were required before insulin-induced glucose utilization in septic patients reached a steady state. Therefore, the study of White, et al (48), using a 2-hour infusion, may have underestimated the true glucose utilization rate in the septic patients because there was no proof that a steady state had been obtained. Nonetheless, their conclusion of a peripheral defect may still be correct despite a potential for error in quantitation.

It has been reported previously that glucose production in septic patients is suppressed only 40% by glucose infused at 4 mg/kg•min (50, 51), and that the suppressive effect of glucose infusion on glucose production is diminished after burn injury as well. However, the glucose-induced plasma insulin concentration increases to only 35 to 50 uU/ml during glucose infusion at 4 mg/kg•min, whereas the plasma insulin concentration during the euglycemic clamp in the present study was a minimum of 300 uU/ml and ranged as high as 2,000 uU/ml. Thus, although there seems to be a diminished effectiveness of physiological levels of insulin to suppress endogenous glucose production in both sepsis and burns, this study indicates that this ineffectiveness can be overcome by sufficiently high insulin concentration. The stimulation of

potassium uptake by insulin requires that insulin bind to its specific receptor and prompt intracellular events which activate the Na-K ATPase (52). Therefore, the present data indicate that, because the septic patients avidly took up potassium in the presence of insulin, the binding of insulin to functional membrane-bound receptors is not impaired. Further support for unimpaired insulin binding to its receptor in septic patients is the fact that insulin-mediated suppression of lipolysis is intact (53). Therefore, the defect in glucose clearance during sepsis seems more likely to be caused by a "post-receptor" defect in the metabolism of glucose (54). However, the indirect calorimetry data do not provide much support for the hypothesis of an impairment in glucose oxidation in sepsis. The total rate of glucose oxidation during the clamp was modestly (although not significantly) lower in sepsis, but the overall energy expenditure was also lower in the septic patients than in the patients with burns. Consequently, the requirement for energy production from glucose was less in the septic patients. Thus, when expressed as a percent of total energy expenditure derived from the oxidation of glucose, there was no difference between the two groups and the normal volunteers. Furthermore, in the septic group, 51% $\pm$ 5.8% of glucose uptake was oxidized, whereas only 42% $\pm$ 5.7% of uptake was oxidized in the burn patients. The latter value is comparable to the rate in normal volunteers (55). Because less than half of glucose uptake involves oxidation, it is reasonable to consider the major pathway of glucose disappearance, glycogen synthesis, to be the potential site of impaired activity. Although impaired glycogen synthesis has not been studied in humans, it has been reported in endotoxic rats (56).

The apparently normal insulin-stimulated glucose uptake in non-septic burn patients is perhaps the most surprising finding of this study. These results seem to contradict those we (37) and others (38, 57) have published previously. One possible explanation is that the exaggerated glucose utilization of the burn wound itself corrects for the diminished uptake by other peripheral tissues such as skeletal muscle. Thus, whole-body glucose clearance would not differ from that of controls, although the contribution of individual tissues had changed. However, the fact that burn patients who became septic more closely resembled other septic patients than non-septic burn patients supports the theory that the burn wound does not increase whole-body glucose utilization sufficiently to mask insulin unresponsiveness in other tissues. A more likely explanation is that previous studies, (e.g., Wolfe, et al) (37) have focused primarily on the stimulatory effect of lower physiological concentrations of insulin, whereas in this study pharmacological levels of insulin were achieved. In addition, the studies performed by Black, et al (38, 57) involved 2-hour clampings; Figure 3 shows that two hours is insufficient time to reach a steady state in glucose uptake in severely injured patients. Finally, our "control" subjects were bedrested volunteers, who had a degree of insulin resistance compared with the value before bedrest. The difference in response between septic and burn patients at comparable (maximal) insulin levels suggests that there may be a unique feature of bacterial sepsis that induces peripheral resistance to the action of insulin to stimulate glucose uptake. Alternatively, it is possible that the septic patients were simply sicker than the uninfected patients and their altered glucose metabolism reflected a more intense generalized stress response than

was present in the uninfected burn patients. These two possibilities will be difficult to distinguish, as it is almost inevitable that a severely septic patient will have greater metabolic and physiological stress than a non-septic injured patient, regardless of the severity of the injury.

Evidence indicates that resistance to the normal protein anabolic effect of insulin may be an important mechanism leading to net catabolism in severe injury or sepsis (58, 59, 60, 61). A general dysfunction of insulin is suggested by the failure of insulin to exert its normal hypoglycemic action (62). It has been proposed that the failure of insulin to normally stimulate glucose uptake and oxidation could lead to protein catabolism indirectly, as a consequence of a peripheral energy deficit. Another possible scenario is that because of the inability of insulin to restrain the stimulatory effect of glucagon on the rate of glucose production and gluconeogenesis, due to the increased plasma glucagon to insulin molar ratio (G/I ratio), there is an increased rate of protein breakdown to supply amino acids as substrates to fuel the accelerated rate of gluconeogenesis (63, 64). Alternatively, the recent work of Hasselgren, et al (58) indicates that in the skeletal muscle of septic rats there is an impairment of the direct action of insulin to inhibit protein breakdown and stimulate synthesis.

In contrast to these findings, other indirect lines of evidence suggest that the protein response to insulin may be reasonably intact. For example, we have previously reported that inhibition of endogenous insulin secretion in severely burned patients elicited a marked increase in alanine flux, indicative of an increased rate of peripheral nitrogen release, hence of protein breakdown, thereby suggesting that the ability of basal insulin to restrain

protein breakdown was intact (65). This is supported by the in vitro findings of Odessey and Parr (66) who reported no impairment in the ability of physiological concentrations of insulin to stimulate protein synthesis and inhibit breakdown in the soleus muscle of burned rats. Furthermore, the results of the previous section indicated insulin action on potassium uptake in patients was intact, despite resistance to the hypoglycemic action of insulin, and we have previously shown the hypothesis investigated in this section is that the normal anabolic effect of insulin on protein metabolism is maintained in burned and/or septic patients who are otherwise "insulin resistant". We have tested the maximal biological effectiveness of insulin on the absolute and net rates of protein breakdown using the hyperinsulinemic euglycemic clamp technique and infusions of stable isotopes of leucine and urea. The maximal biological effectiveness of insulin was assessed because this approach is most likely to uncover a "post-receptor" defect in insulin action (67), and this has been postulated to be the basis of insulin resistance in severely burned patients (68). To assess more directly the possible role of a defect in glucose oxidation or energy deficit as a mediator of the net protein catabolic response we have evaluated in one group of patients the response of protein metabolism to the hyperinsulinemic clamp with and without the simultaneous administration of dichloroacetate (DCA). DCA stimulates pyruvate dehydrogenase (PDH) activity and thus glucose oxidation.

METHODS:

Patients:

Eight severely burned and 8 septic patients who were admitted to the Burn, Surgical and Medical Intensive Care Units of The University of Texas

Medical Branch in Galveston, Texas were studied. Characteristics of the individual patients are summarized in Tables 14 and 15. The seven male and one female burn patients ranged in age from 16 to 42 year and had a mean burn size of 65% of total body surface area (TBSA) of which 35% TBSA was third degree. At the time of study, between postburn days 4 to 17, all patients were in a hypermetabolic state as indicated by comparing their basal metabolic rate to expected values for normal subjects of similar age and size. They had a mean heart rate of 117 $\pm$ 4 beats/min and systolic blood pressure of 126 $\pm$ 4 mmHg. Core temperature ranged from 37.3 to 39.0 degree C (Table 14). Clinical treatment of all patients was comparable, with early excision of the burn wound and grafting as described in detail elsewhere (65).

Sepsis was assessed by the presence of infection in blood, cerebrospinal fluid, urinary tract, peritoneum, lung, wound or abscess, coupled with fever, leukocytosis, thrombocytopenia, altered mental status and hyperdynamic cardiovascular status (33, 69). The one female and seven male septic patients ranged in age from 25 to 45 year (Table 15). According to the classification of Elebute and Stoner (33) they had a mean sepsis score of 17 $\pm$ 1 at the time of study, and were in a hyperdynamic state with a mean cardiac index of 6.2 $\pm$ 0.3 l $m^{-2} \cdot min^{-1}$, heart rate of 112 $\pm$ 3 beats/min, systolic blood pressure of 127 $\pm$ 7 mmHg, and core temperature ranging from 37.3 to 39.2 degree C.

Patients had no personal history of any metabolic diseases and at the time of study had normal renal function as judged by serum creatinine levels and urine output.

The experimental protocol was approved by the Institutional Review Board of The University of Texas Medical Branch and informed consent was obtained

after the nature, purpose and potential risks of the study were explained in detail to the patient and/or relatives in the presence of a patient advocate.

Materials:

The isotopes infused as tracers were 1-^{13}C-leucine, 99% enriched, obtained from Tracer Technologies, Inc. (Somerville, MA) and $^{15}N_2$-urea, 99.7% enriched, from Merck Isotopes (Montreal, Canada). Dichloroacetate (DCA) was obtained from Continental Trading Company Organics (Atlanta, GA). Sterile solutions of the isotopes and DCA were prepared in 0.45% saline and passed through a 0.22 um filter (Millipore Corp., Bedford, MA) into sterile evacuated containers. For each infusion an aliquot of the infusate was analyzed for the exact isotope concentration in order to calculate the actual infusion rate for each study. Insulin solution (2 U/ml) for infusion was prepared by adding regular human insulin (Novolin, E. R. Squibb & Sons, Princeton, N.J.) to normal saline containing 2.5 ml of the patient's blood. The isotopes and insulin solutions were infused using a Bard Model 2716 Syringe Infusion Pumps (Bard Med Systems, South Natick, MA).

Experimental Design:

There were two different experimental protocols (Figure 12); isotopes of leucine and urea were infused. The rate of appearance (Ra) of leucine was used as an index of the absolute rate of protein breakdown and its rate of oxidation as an index of net protein catabolism. Because all studies were performed in fasted patients, leucine (being an essential amino acid) could only have appeared from protein breakdown. Its flux can therefore be considered a good index of the absolute rate of protein breakdown. Second, because of its essentiality leucine it cannot be resynthesized after oxidation

and is therefore unavailable to the body for protein synthesis. For this reason the rate of leucine oxidation can be considered as an index of net protein loss. Because urea synthesis represents the final pathway of nitrogen disposal, its rate of production was also used as a more direct indicator of net protein catabolism. The isotopic technique was used to quantify urea kinetics to overcome difficulty in accurately determining total urea excretion.

Protocol 1:

The primary aim of this protocol was to test the maximum biological effectiveness of insulin to restrain the absolute and net rates of protein breakdown in severely burned and in septic patients. After the bicarbonate pool was primed with 1.7 umol/kg of $NaH^{13}CO_3$, a primed-constant infusion of $^{15}N_2$-urea (infusion rate of 0.14 umol$\cdot kg^{-1}\cdot min^{-1}$, prime 84 umol/kg) and 1-^{13}C-leucine (infusion rate of 0.25 umol$\cdot kg^{-1}\cdot min^{-1}$, prime 15 umol/kg) was started and maintained throughout an 8 hour study period (Figure 1). After 3 hours of isotope infusion only (basal period), the insulin pool was primed with a bolus dose, calculated to raise plasma concentration to 1000 uU/ml, followed by a constant infusion of insulin at the rate of 500 mU/$m^{-2}\cdot min^{-1}$ for 5 hours. The insulin infusion was accompanied by a variable infusion of 20% D-glucose solution at a rate sufficient to maintain plasma glucose concentration at 90 mg/dl. During this hyperinsulinemic period plasma glucose level was closely monitored at 5 to 10 minute intervals using the glucose oxidase reaction (Glucose Analyzer 2, Beckman Instruments, Inc., Fullerton, CA). Serum potassium levels were monitored at 30-min intervals and maintained within the normal range by infusing a 50 mEq/l KCl solution. The rate of delivery of maintenance fluids

was appropriately adjusted during the study period in order to maintain a comparable fluid delivery rate and a constant state of hydration.

Blood and expired air samples were collected before the isotope infusions started and at 15-minute intervals during the last 90 minutes of the basal and hyperinsulinemic periods. During the course of a study the patients' metabolic rate (O_2 consumption, CO_2 production) was also measured in both periods, with a Horizon Metabolic Cart (Beckman Instruments).

Protocol 2:

Eight patients (5 burned, 3 septic, Tables 14 and 15), who had already participated in protocol 1, were studied for a second time in this protocol. The aim of this protocol was to determine the relationship between the rate of glucose oxidation and leucine and urea kinetics during the hyperinsulinemic clamp. This was accomplished by studying each patient twice. The first study was as described for protocol 1. The same protocol was then repeated, but at the start of the hyperinsulinemic period and then 2.5 hours later, DCA was infused at a rate sufficient to deliver a dose of 35 mg/kg over 30 min. DCA was given in order to stimulate PDH activity and thereby increase the fraction of pyruvate entering the tricarboxylic acid cycle for ultimate oxidation.

Analysis of Samples:

Calculations:

The description of the methods of analysis and calculations have been discussed in earlier sections of this report.

Statistical Analysis:

Dunnett's (the paired) t-test was used to compare basal data with data obtained during the hyperinsulinemic period (protocol 1) or the hyperinsulin-

emic plus DCA period (protocol 2). To compare data from burned patients with data from septic patients the non-paired t-test was used. The paired t-test was also used for the comparison of the results during the clamp and with those during clamp plus DCA (protocol 2). Results are presented as mean ± SEM.

RESULTS:

Protocol 3:

Values for the normal post-absorptive state were obtained from a control study performed concurrently in healthy normal volunteers and described in the earlier section under bedrest.

In the burned and septic patients the insulin infusion caused comparable increases in plasma insulin levels from within the normal range (9.6 ± 1.9 and 8.6 ± 1.1 uU/ml respectively) to 1298 ± 347 and 1213 ± 229 uU/ml respectively. Mean basal glucose concentration was identical in both groups, 108 ± 6 mg/dl, and during the insulin infusion was clamped at exactly 91 ± 1.1 and 91 ± 0.7 mg/dl respectively in each group. This required a glucose infusion rate of 11.5 ± 0.7 $mg \cdot kg^{-1} \cdot min^{-1}$ in burned patients and 6.5 ± 1.2 $mg \cdot kg^{-1} \cdot min^{-1}$ in septic patients, (significantly different, $p < 0.01$).

In burned and septic patients basal leucine Ra was 5.15 ± 0.24 and 4.08 ± 0.22 $umol \cdot kg^{-1} \cdot min^{-1}$ respectively (Table 16) ~ 2 and 1.5 times the control value of 2.78 ± 0.16 $umol \cdot kg^{-1} \cdot min^{-1}$, indicating a significant stimulation of the absolute rate of protein breakdown in both groups of patients. Nonetheless, the hyperinsulinemic clamp reduced Ra leucine in both patient groups to a comparable extent as that in controls (Table 16). However, whereas in absolute terms the decrease in leucine Ra elicited by hyperinsulinemia in the

septic patients (1.09 ± 0.09 umol•kg^{-1}•min^{-1}) (Figure 13) was comparable to that of the controls (1.15 ± 0.12 umol•kg^{-1}•min^{-1}) and even greater ($p < 0.01$) in the burns, (1.76 ± 0.16 umol•kg^{-1}•min^{-1}), the insulin infusion still failed to reduce leucine Ra to a normal level in either group, (3.39 ± 0.14 in burns, 2.99 ± 0.19 in sepsis versus 1.64 ± 0.08 umol•kg^{-1}•min^{-1} in controls, Table 16).

The basal rates of leucine oxidation in the burned, (1.6 ± 0.14 umol•kg^{-1}•min^{-1}), and septic patients (1.25 ± 0.15 umol•kg^{-1}•min^{-1}), were more than 2 times the control rate of 0.61 ± 0.07 umol•kg^{-1}•min^{-1} (Table 16). As in the case of Ra leucine, the insulin infusion significantly reduced ($p < 0.05$) leucine oxidation in both groups of patients (Table 16) by amounts comparable to the decrease in the control group (from 0.61 ± 0.01 to 0.32 ± 0.01 umol•kg^{-1}•min^{-1}). As with leucine flux, however, the insulin infusion failed to reduce leucine oxidation in either patient group to the value for controls (0.32 ± 0.03 umol•kg^{-1}•min^{-1}). Compared to control values, basal urea Ra was elevated by ~ 100 and 50% in the burned and septic patients respectively (Table 16). Consistent with the effect of insulin on reducing leucine flux and oxidation, the insulin infusion elicited significant and comparable reductions ($p < 0.05$) in urea Ra in both groups of patients (Table 16).

In both groups of patients the insulin infusion elicited significant reductions ($p < 0.05$, $p < 0.01$) in the concentrations of all plasma amino acids (Table 17).

Protocol 4:

The insulin infusion alone caused plasma insulin levels to increase from a basal value of 9.6 ± 1.9 uU/ml to 1132 + 143 uU/ml. The simultaneous admin-

istration of DCA did not affect this response as basal insulin increased from 9 ± 1.1 uU/ml to 1165 ± 196 uU/ml. Mean basal glucose concentration was 90 ± 4 and 100 ± 7 mg/dl, respectively, during the two studies. During the insulin infusion, glucose was clamped at 90 ± 1.3 mg/dl and when DCA was also infused it was clamped at about the same level, 91 ± 0.5 mg/dl. This required a glucose infusion rate of 9.7 ± 1.2 mg/kg•min in the hyperinsulinemic clamp alone (protocol 1) and 10.1 ± 1.1 mg/kg•min when DCA was also given (protocol 2). Since endogenous glucose production is totally suppressed during the hyperinsulinemic clamp period, the rate of glucose infusion is equal to the rate of glucose uptake, suggesting that DCA administration had no additive effect on the rate of glucose uptake during the hyperinsulinemic clamp. In response to the clamp (± DCA) the rate of $\dot{V}CO_2$ production and the respiratory quotient (RQ) increased significantly in both studies (Table 18). Basal RQ increased significantly from 0.80 ± 0.02 to 0.92 ± 0.02 during the clamp without DCA ($p < 0.01$), and the additional administration of DCA caused basal RQ to increase even further from 0.78 ± 0.01 to 0.97 ± 0.02 ($p < 0.01$), confirming the stimulatory action of DCA on PDH. This is shown in Figure 14 where hyperinsulinemia and hyperinsulinemia plus DCA cause the basal rate of glucose oxidation to increase significantly ($p < 0.01$) from 1.61 ± 0.24 to 4.60 ± 0.62 $mg \cdot kg^{-1} \cdot min^{-1}$ and from 1.15 ± 0.30 to 5.70 ± 1.0 $mg \cdot kg^{-1} \cdot min^{-1}$ respectively. DCA increased the percent of glucose uptake oxidized during the clamp from 45.5 ± 5.5% to 53.5 ± 4.8% ($p < 0.05$). In absolute terms the increase in glucose oxidation rate when DCA was also given, 4.5 ± 0.73 $mg \cdot kg^{-1} \cdot min^{-1}$ was significantly greater ($p < 0.05$) than the increase observed during the clamp alone, 2.98 ± 0.65 $mg \cdot kg^{-1} \cdot min^{-1}$ (Figure 14). Preferential oxidation of pyruvate

during DCA treatment is also suggested by the fact that there were greater decreases ($p < 0.01$) in both plasma lactate and alanine concentrations during clamp plus DCA than clamp alone (Table 19).

Both the hyperinsulinemic clamp and the clamp plus DCA elicited significant and comparable reductions in leucine Ra from a basal value of 4.59 $\pm$ 0.34 to 3.05 $\pm$ 0.19 umol$\cdot$kg$^{-1}\cdot$min^{-1} and from 4.55 $\pm$ 0.34 to 2.54 $\pm$ 0.27 umol$\cdot$kg$^{-1}\cdot$min^{-1} respectively, (Table 20). In absolute terms although the reduction in leucine Ra due to the clamp plus DCA (decrease of 2.01 $\pm$ 0.34 umol$\cdot$kg$^{-1}\cdot$min^{-1}) was 30% greater, it was not significantly different ($p = 0.076$) from the reduction of 1.54 $\pm$ 0.18 umol$\cdot$kg$^{-1}\cdot$min^{-1} due to the clamp alone. Six out of 8 patients, however, responded with a greater decrease in leucine Ra (due to the DCA) which was significantly different ($p < 0.05$) from the decrease in leucine Ra elicited by the clamp alone. Similarly the rates of leucine oxidation and urea production were suppressed comparably by insulin, regardless of the presence of DCA (Table 20).

As in protocol 1, the hyperinsulinemic clamp elicited significant reductions in the plasma concentrations of all amino acids (Table 21). DCA did not markedly affect this response, although the decreases in aspartic acid, glycine and histidine, failed to reach a level of significance when DCA was given, and the decrease in alanine concentration, 159 $\pm$ 13 umol/ml, was twice the magnitude of the decrease 79 $\pm$ 8 umol/ml ($p < 0.01$) due to the clamp alone (Table 19).

DISCUSSION:

The primary aim of this study was to determine if there was a defect in insulin's action on protein metabolism in burn injury and sepsis and the role

of glucose oxidation in mediating the protein catabolic response in these patients. We found that hyperinsulinemia reduced leucine flux and rate of oxidation to the same extent as in normal controls but failed to normalize either. These results suggest that there is no impairment in the maximum biological effectiveness of insulin to inhibit the absolute and net rates of protein breakdown in severe burn injury and sepsis. However, the failure of hyperinsulinemia to normalize leucine kinetics means that a possible role of insulin resistance in mediating the elevated basal rate of protein catabolism cannot be ruled out in these patients. Secondly, since the increase in glucose oxidation during the hyperinsulinemic clamp failed to normalize leucine and urea kinetics in both groups of patients, and since the further stimulation of the rate of glucose oxidation by DCA administration failed to significantly affect either leucine or urea kinetics, it can be concluded that the protein catabolic response in these patients was not mediated via a defect in glucose oxidation or a deficit in peripheral energy supply secondary to an impairment in glucose oxidation. Finally, because endogenous glucose production was totally suppressed during the hyperinsulinemic clamp (69), the failure to normalize leucine and urea kinetics during the clamp in these studies suggests that an increased utilization of amino acids to fuel an accelerated rate of gluconeogenesis is not the major cause of the stimulated rate of net protein catabolism in burn injury and sepsis.

The present finding that the absolute and net rates of protein breakdown are markedly stimulated in severe burn injury and sepsis is in agreement with our previous reports (70, 71) and those of others (63, 64, 65). The suppression of leucine flux and oxidation by hyperinsulinemia has been reported by us

and other investigators both in normal volunteers (72, 73) and in severely traumatized patients (74), suggesting that the protein sparing effect of insulin demonstrated in severely traumatized patients by others (65) is due to the ability of insulin to restrain both the absolute and net rates of protein breakdown. Since the extent to which hyperinsulinemia inhibited the absolute and net rates of protein breakdown in each group of patients in this study was comparable to its effect in normal volunteers (72), it can be concluded that there is no impairment in the maximum biological effectiveness of insulin to restrain absolute and net protein catabolism in burn injury and sepsis. These results are in agreement with the in vivo findings of Inculet, et al (59) in post-operative trauma patients, and the in vitro findings of Odessey and Parr (66) in the soleus muscle of burned rat and Ryan, et al (61) in the skeletal muscle of septic rabbit, that insulin administration suppresses net protein breakdown but fails to reduce it to control or pre-operative levels. In the only other study employing the hyperinsulinemic euglycemic clamp to investigate the role of insulin in mediating the protein catabolic response to trauma, Brooks, et al (74) reported a four-fold suppression of amino-N efflux from the skeletal muscle bed of injured patients to the level seen in normal control subjects. This response of amino-N efflux to the hyperinsulinemic clamp was, however, far more pronounced than that observed in our study or in the study of Inculet, et al (59). Furthermore, both our present study and that of Inculet, et al (59) failed to demonstrate a normalization of leucine or amino-N flux, or of leucine oxidation in response to the insulin infusion. On the other hand, only the in vitro study of Hasselgren, et al (58) reported

a failure of hyperinsulinemia (1000 uU/ml) to elicit any suppression of the elevated rate of muscle protein breakdown in septic rats.

The normal responsiveness of protein kinetics to hyperinsulinemia in severe burn injury and sepsis as demonstrated in this study does not rule out a possible role of insulin resistance in mediating the elevated basal protein catabolism in these patients. The fact that the same degree of hyperinsulinemia failed to reduce the absolute and net rates of protein catabolism in both groups of patients to the extent seen in normal volunteers, supports the contention of Inculet, et al (59) that insulin resistance or factors acting via insulin resistance may be responsible for mediating the net protein catabolic response to injury and sepsis. Alternatively it could be argued that the ability of insulin to reduce but not normalize the absolute and net rates of protein breakdown could be due to some of the proteins whose breakdown is markedly accelerated not being responsive to insulin under any condition.

Our results also fail to support any possible role of an impairment in glucose oxidation and/or a deficit in peripheral energy supply in mediating the stimulated rates of absolute and net protein catabolism in severe burn injury and in sepsis. The concept that there is an increase in muscle protein breakdown to supply amino acids as substrates in order to correct a deficit in peripheral energy availability arose out of the combined in vitro work of Ryan, et al (60, 61) and in vivo findings of O'Donnell, et al (75, 76). Based on their findings that septic rats had a markedly reduced blood free fatty acid (FFA) concentration, a reduced rate of uptake and oxidation of glucose by muscle (due to an inhibition of muscle PDH activity) coupled with an increased rate of leucine oxidation, and decreased rate of muscle protein synthesis,

Ryan, et al (60, 61) proposed that in sepsis there is a reduced mobilization of fat without a concomitant increase in glucose oxidation in muscle, resulting in an inadequate supply of metabolic fuel for muscle. This in turn promotes amino acid oxidation by muscle to meet cellular energy requirements (60, 61). This proposal received further in vivo support from the work of O'Donnell, et al in the septic pig (75) and in septic patients (76) and of Clowes, et al (63) in severely traumatized and septic patients. However, our results show that although there is an impairment in the ability of septic (but not burned) patients to take up glucose (69), once the glucose has entered the cell there is no defect in the ability of burned or septic patients to oxidize glucose (Ref. 69 and Figure 14) as the percent of glucose uptake oxidized is the same (40%) in burns, sepsis and controls in both the basal state and during the clamp (69). Also, in the past we have shown that the basal rate of uptake of glucose and the rate of lipolysis in severely burned and septic patients are markedly elevated compared to control values (62, 65, 77). Thus in spite of the fact that during the hyperinsulinemic clamp the increase in the amount of glucose oxidized in the burn patients, 3.2 $mg \cdot kg^{-1} \cdot min^{-1}$, was more than twice the increase in septic patients, 1.3 $mg \cdot kg^{-1} \cdot min^{-1}$ (69) the reduction in the flux and rate of oxidation of leucine in both groups of patients were comparable (Table 16), suggesting that the reduction in the absolute and net rates of protein breakdown during the hyperinsulinemic clamp was probably due to the direct protein sparing action of insulin and not to an increase in energy availability resulting from the stimulated rate of glucose oxidation. Secondly, since it can be calculated from our indirect calorimetry data (Table 18) that the rate of fat oxidation remains unchanged when DCA is

administered during the hyperinsulinemic clamp, the further increase in glucose oxidation, of 1.5 $mg \cdot kg^{-1} \cdot min^{-1}$ in response to the DCA treatment (Figure 14) should have spared an equivalent amount of amino acid from being oxidized. This should translate into a decrease in the rate of urea production. In reality, however, the additional stimulation of glucose oxidation by DCA did not elicit any additional decrease in the rate of production of urea (Table 20).

Differences in experimental approaches may explain why studies such as those of O'Donnell, et al (75, 76) and Clowes, et al (63) have reported a deficit in peripheral energy supply in sepsis, whereas our studies employing stable isotope tracer techniques have consistently indicated the absence of an impairment in glucose oxidation (62, 65) and an increased rate of lipolysis and fat oxidation in severely burned or septic patients (77, 78), sufficient to meet the stimulated basal energy requirements of severe trauma (79). A potential flaw of studies estimating energy availability by measuring substrate balance across an organ or tissue bed, as was done in the earlier studies (63, 75, 76), is that such net balance techniques do not take into account substrates such as glycogen and FFA which can be released within the tissue bed or organ and oxidized for energy without being released into the blood compartment. Hence A-V balance measurements will always underestimate the actual amount of substrates available to the organ or tissue bed for oxidation.

It should be mentioned that the further decrease in leucine Ra elicited by the addition of DCA, although not statistically significant, was 30% greater than the decrease elicited by the clamp alone (Table 20), suggesting

that the increased glucose oxidation (or energy supply) elicited a further reduction in the absolute rate of protein breakdown. However, because the change in leucine oxidation rate elicited by both the clamp and the clamp plus DCA was identical (Table 20) this merely means that the non-oxidative disposal rate of leucine (or protein synthesis) was also suppressed to a greater extent when the DCA was given. That is, in terms of net protein catabolism the decrease was the same in both situations. However, protein turnover (breakdown plus synthesis) rate was suppressed when DCA was also administered.

Another point that warrants discussion is the fact that in vitro studies have shown that DCA by itself suppresses leucine oxidation in muscle preparations (79, 80) and elicits an increased release of BCAA's from the perfused hindquarter of fasted rats (81). Translated to the situation in vivo in the whole-body, one would therefore predict that a direct DCA effect on leucine kinetics will cause a further decrease in leucine oxidation (adding to the decrease elicited by hyperinsulinemia alone) and a lesser decrease in leucine flux compared to the decrease elicited by the clamp alone. The fact that neither of these observations were made rules out any direct DCA effect on leucine kinetics. It should also be pointed out that the dose of DCA used to elicit a response in leucine metabolism in both the in vitro studies (79, 80) and the perfused rat hindquarters study (81) was six times the dose given in the present study.

Lastly, our results do not support the role of an accelerated rate of gluconeogenesis as a major mediator of the increased net protein catabolism in severe burn injury and sepsis, as proposed by some investigators (63, 64). The increased glucagon/insulin molar ratio in stressed patients increases

hepatic glucose output (80), in part by stimulating the rate of gluconeogenesis (64). It has been argued that this, in turn, causes an increase in peripheral protein breakdown in order to supply amino acids as substrates to fuel this accelerated rate of gluconeogenesis (64). Our results, however, show that during the hyperinsulinemic clamp endogenous glucose production is totally suppressed (69), suggesting that hepatic gluconeogenesis is also totally suppressed; yet the absolute and net rates of protein breakdown are still elevated and fail to reach a normal control value. This would suggest that the accelerated rate of gluconeogenesis is not solely responsible for the increased absolute rate of protein breakdown. On the other hand, accelerated irreversible deamination of amino acid precursors for gluconeogenesis presumably plays a role in the high net rate of protein breakdown in severely burned and septic patients.

The important clinical finding in this study is that the stimulated rate of net protein catabolism in severely burned and septic patients is not mediated via a defect in glucose metabolism or a deficit in energy supply. Hence therapy designed specifically to reduce protein loss by stimulating glucose uptake and oxidation will have a minimal sparing effect only. On the other hand, the retained protein anabolic effect of insulin is obviously of potential benefit to the patient in terms of anabolism. However, because of documented side effects of excessive rates of glucose administration that must be given to avoid hypoglycemia when large amounts of exogenous insulin is given, further studies to delineate more specific dose-effectiveness levels of insulin are in order.

ROLE OF BASAL INSULIN IN THE REGULATION OF PROTEIN KINETICS AND ENERGY METABOLISM IN SEPTIC PATIENTS

INTRODUCTION:

The hypermetabolic response to severe burn injury and sepsis is clinically characterized by hyperglycemia, increased resting energy expenditure and massive loss of body protein (82-84). These metabolic changes are believed to be mediated, at least in part, by elevated catabolic hormones including catecholamines, glucagon, and cortisol (85-87). However, whereas combined infusion of cortisol, glucagon and epinephrine in non-stressed subjects initiated and maintained metabolic responses similar to those of stressed clinical states, (88, 89), the magnitude of the changes in volunteers was less than that observed in patients. The failure of the "triple hormone" infusion in healthy volunteers to mimic the catabolic response in patients may have been due to an effective counterregulatory response to insulin. In stressed patients, on the other hand, insulin concentration is generally not elevated and insulin action also appears to be diminished, at least in terms of its hypoglycemic effect (82, 90).

Insulin is a key anabolic hormone and plays an important role in the regulation of substrate metabolism. The concept of "insulin resistance" is therefore attractive in explaining the catabolic state following major trauma or sepsis, but this is undoubtedly an oversimplification. Whereas the suppressive effect of elevated concentrations of insulin on hepatic glucose production is diminished in stressed patients, basal insulin, nonetheless, plays an important role in restraining glucose production (86). More importantly, in both burned and septic patients who were resistant to the

hypoglycemic action of insulin, hyperinsulinemia reduced leucine Ra and oxidation rate and urea production rate as effectively as in normal controls (91), (previous section).

Although we have established the role of basal insulin and of hyperinsulinemia on glucose metabolism (82, 86), we have only assessed the role of hyperinsulinemia on protein kinetics (91). Since there is controversy regarding the ability of physiological levels of insulin to restrain protein breakdown and to stimulate protein synthesis in trauma and sepsis (92, 93), in the present study we investigated the role of basal insulin on protein kinetics and energy metabolism in septic patients. 1-^{13}C-leucine was used to model protein kinetics, and indirect calorimetry was used to calculate substrate oxidation rates and energy expenditure. The role of basal insulin was assessed by comparing values before and during the reduction of concentration by somatostatin infusion. Somatostatin also reduces glucagon in septic patients, but the concentration remains markedly elevated (86).

METHODS:

Patients:

Eight septic patients (six male, two female, aged 22-58 year) were studied (Table 22). Four patients developed sepsis as a complication of severe burn injury. They had a mean burn size of 54% total body surface area (TBSA) (range 50-60% TBSA), of which 5% was third degree (range 0-18% TBSA). The other four septic patients were primarily diagnosed as acute pancreatitis, incarcerated ventral hernia, multiple trauma and gun shot wound respectively, and became septic during hospitalization.

Sepsis was diagnosed as documented infection in cerebrospinal fluid, blood, lung, peritoneum, urinary tract, wound or abscess; coupled with fever, leukocytosis, thrombocytopenia, altered mental status and hyperdynamic cardiovascular status. According to the classification of Elebute and Stoner (33), their mean sepsis score was 15 $\pm$ 2 at the time of the study. They had a mean heart rate of 112 $\pm$ 6 beats/min, respiratory rate of 24 $\pm$ 2/min, systolic blood pressure of 123 $\pm$ 0 mmHg, which indicated a hyperdynamic state. Body core temperature was 38.0 $\pm$ 0.3 degree C. The patients had no personal history of metabolic diseases. They all survived the septic episodes.

Enteral nutrition was stopped six hours before the start of the study, and four hours before the study intravenous nutrition solutions and insulin were also withheld. During the period of fasting before the start of the study 0.45% saline was administered to maintain a comparable fluid delivery rate and a normal state of hydration. The above conditions were well controlled except for case H, who received D5W infusion at a rate of 150 ml/hr to prevent hypoglycemia.

The experimental protocol was approved by the University of Texas Medical Branch Institutional Review Board. The nature, purpose and risk of the study were explained in detail to all patients and/or relatives in the presence of a patient advocate, and informed consent was obtained.

Materials:

L-(1-^{13}C)-leucine, 99% enriched, was obtained from Merck Isotope (Montreal, Canada). The isotope was dissolved in normal saline and passed through a 0.22 um filter (Millipore Corp., Bedford, MA) into sterile evacuated containers for intravenous infusion. For each infusion an aliquot of the

infusate was analyzed for exact isotope concentration. $NaH^{13}CO_3$, 99% enriched, was obtained from INC Biomedicals (Costa Mesa, CA), and dissolved in a solution of 500 umol/ml in 0.01N NaOH. Somatostatin, obtained from Bachem (Torrance, Ca) was dissolved in sterile normal saline immediately before the experiment.

Experimental Design:

Isotope tracers were infused into each patient for 4 hours, beginning around 9 a.m. All patients had an arterial and a venous line already in place for therapeutic purposes. The pre-existing venous line was used for infusion and the arterial line was used for blood sampling. There were two experimental periods (Figure 15). During the first two hours basal values were determined. A primed-constant infusion of L-(1-^{13}C)-leucine (infusion rate of 0.25 $umol \cdot kg^{-1} \cdot min^{-1}$, prime, 15 umol/kg) and a bolus injection of $NaH^{13}CO_3$ (1.2 umol/kg) were given after background samples of blood and expired CO_2 were collected. After two hours of isotope infusion only, somatostatin infusion was started at 0.15 $ug \cdot kg^{-1} \cdot min^{-1}$ without disruption of the ^{13}C-leucine infusion. Blood samples were drawn every 15 min before 60-120 min of the basal period, and every 20 min throughout somatostatin infusion. Expired breath samples were collected at times corresponding to blood samples in order to determine $^{13}CO_2$ enrichment. Total CO_2 production ($\dot{V}CO_2$) and O_2 consumption ($\dot{V}O_2$) were determined by indirect calorimetry using a Horizon Metabolic Cart (Beckman Instrument, Fullerton, CA) at multiple intervals, and the readings averaged for each period.

Analysis of samples and calculations were as described in the earlier sections.

Statistical Analysis:

Paired t-test was used to compare the leucine kinetics and indirect calorimetry parameters observed during somatostatin infusion with the basal values. Results are presented as mean ± SEM for values calculated during isotopic and physiological steady states.

Results:

The patients had markedly elevated plasma glucagon levels of 744 ± 381 pg/ml (normal value of 60 pg/ml), while insulin levels (10 ± 4 uU/ml) were within the normal range (5-20 uU/ml), except in case H (26 uU/ml) who was receiving glucose at 1.42 $mg \cdot kg^{-1} \cdot min^{-1}$ before and during the basal period (Figure 16). The infusion of somatostatin resulted in a reduction of insulin level by 52% to 5 ± uU/ml, and glucagon level by 64% to 273 ± 151 pg/ml, which was still more than four times the normal value. During the basal period, glucose concentration was stable, but decreased rapidly after somatostatin infusion started (Figure 17). Five of the eight patients required exogenous glucose at 0.79 ± 0.39 $mg \cdot kg^{-1} \cdot min^{-1}$ to maintain euglycemia, whereas in three subjects the glucose concentration was allowed to drop to about 50 mg/dl. Regardless of whether or not euglycemia was maintained by glucose infusion, the leucine kinetics and metabolic response of all eight patients were always in the same direction.

The basal rates of leucine appearance (Ra) and oxidation were 3.90 ± 0.21 and 0.95 ± 0.08 $umol \cdot kg^{-1} \cdot min^{-1}$ respectively (Table 23), which are significantly greater ($p < 0.01$) than the values of 2.79 ± 0.17 and 0.61 ± 0.07 $umol \cdot kg^{-1} \cdot min^{-1}$ in normal post-absorptive volunteers. During somatostatin infusion, Ra leucine was unchanged (3.85 ± 0.18 $umol \cdot kg^{-1} \cdot min^{-1}$), but leucine

oxidation increased significantly ($p < 0.01$) from 0.95 $\pm$ 0.08 to 1.18 $\pm$ 0.14 $umol/kg^{-1} \cdot min^{-1}$. This was associated with a significant decrease in the non-oxidative disposal rate from 2.95 $\pm$ 0.18 to 2.67 $\pm$ 0.17 $umol \cdot kg^{-1} \cdot min^{-1}$ ($p < 0.01$), suggesting a decrease in protein synthesis rate, and thus a greater rate of net protein loss.

In the basal period resting energy expenditure (REE) was 1310 $\pm$ 100 $kcal/m^2$ day, which is 151 $\pm$ 8% that of predicted basal energy expenditure (BEE) (Table 24). During somatostatin infusion, both CO_2 production and O_2 consumption rates were significantly greater than in the basal period ($p < 0.05$), resulting in a significantly increased REE (from 1310 $\pm$ 100 to 1505 $\pm$ 128 $kcal/m^2$ day; $p < 0.05$). Fat oxidation increased from 1.72 $\pm$ 0.24 to 2.41 $\pm$ 0.41 $mg \cdot kg^{-1} \cdot min^{-1}$ while carbohydrate oxidation slightly decreased from 1.15 $\pm$ 0.49 $mg \cdot kg^{-1} \cdot min^{-1}$ to 1.31 $\pm$ 0.49 $mg \cdot kg^{-1} \cdot min^{-1}$. When the four burn-septic patients were compared with the other four septic patients all the leucine kinetics and indirect calorimetry parameters exhibited the same changes.

Most plasma amino acid concentrations increased during somatostatin infusion, except hisitidine concentration, which fell. However, only the increases in threonine, serine, glutamine and leucine were significant ($p < 0.05$) (Table 25). Overall there was a significant increase ($p < 0.05$) of total amount of amino acids measured in plasma during somatostatin infusion.

DISCUSSION:

The goal of this study was to assess the role of basal insulin concentration in the regulation of protein kinetics and energy metabolism. Somatostatin was used to decrease insulin concentration, thereby allowing the assessment of the pre-existent role of insulin. Somatostatin also reduced the glu-

cagon concentration, but that did not affect interpretation of the results. The increases in leucine oxidation, protein catabolism, and metabolic rate that were caused by somatostatin would all have been in the opposite direction if the changes were reflecting the pre-existing role of glucagon, since all of these responses are caused by increasing (rather than decreasing) glucagon concentration in normal volunteers (94). The predominance of the change in insulin concentration during somatostatin can probably be attributed to the fact that whereas insulin was reduced to values below normal, glucagon concentration still remained more than four-fold above the normal concentration. Thus, from the responses to somatostatin infusion we can deduce that basal insulin concentration in septic patients effectively stimulates protein synthesis, restrains net protein breakdown, and also that insulin restrains the resting energy expenditure and rate of fat oxidation. Furthermore, the pre-existent role of insulin may have been underestimated by the response to somatostatin infusion, since the simultaneous fall in glucagon may have attenuated some of the changes due to the drop in insulin.

The findings of this study support our previous contention and those of others (95) of an intact anabolic effectiveness of basal insulin in the regulation of protein metabolism in catabolic states in human patients. We previously found in severely burned patients that hypoinsulinemia elicited a marked increase in alanine release that did not occur when basal insulin was maintained, suggesting that the ability of basal insulin to restrain protein loss was intact. These in vivo studies are consistent with in vitro studies using the soleus muscle of the burned rate limb, which confirmed the ability of physiological levels of insulin to stimulate protein synthesis and inhibit

breakdown in a normal fashion (93). Furthermore, we found that the hyperinsulinemic euglycemic clamp procedure suppressed both leucine flux and oxidation in severely burned and septic patients to the same extent as in normal volunteers, even though the septic patients were resistant to the hypoglycemic action of insulin (91).

Because of the parallel and almost proportional decrease in leucine flux and oxidation rate due to hyperinsulinemia in normal subjects (96), non-oxidative disposal of leucine (used as an index of protein synthesis rate) is also decreased, suggesting that increased insulin concentration normally exerts its anabolic effect on protein metabolism primarily via a suppression of protein breakdown (97). The present findings of an increased leucine oxidation rate during hypoinsulinemia suggest that basal insulin exerts its anabolic effect on protein metabolism in septic patients by a different mechanism. That is, basal insulin inhibits leucine (and presumably other amino acid) oxidation and stimulates protein synthesis. The stimulation of incorporation of amino acids into protein could be the direct response to insulin, whereas the decrease in oxidation could be due to a decreased availability (97). Alternatively, decreased oxidation could lead to greater availability of amino acids for protein synthesis. In contrast to the situation with hyperinsulinemia (in patients or volunteers), the basal insulin concentration in these patients seemed to play little role in restraining protein breakdown, since leucine flux (a reflection of breakdown) failed to respond to hypoinsulinemia. However, it is also possible that the pre-existent role of insulin in restraining protein breakdown was obscured by the simultaneous reduction in glucagon. In

this case the question could only be resolved by replacement of basal glucagon, which is technically impractical in this clinical circumstance.

The increase in concentration of most amino acids in these patients after two hours of somatostatin infusion provides additional evidence that hypoinsulinemia reduced protein synthetic rate. The size of an individual amino acid pool is dependent upon the difference between its flux (rate of appearance) and oxidation plus disposal rates. When flux is unchanged, as was the case with leucine during hypoinsulinemia, but oxidation increases, disposal rate will decrease proportionately, if pool size remains constant. If pool size increased, however, as leucine did during hypoinsulinemia, disposal rate will not be just the difference between flux and oxidation rate, but the difference between flux and the sum of oxidation plus the increase in pool size. This means that in the present study since the calculation of non-oxidative disposal was based on the assumption that the free amino acid pool was constant, there was a potential overestimation of non-oxidative leucine disposal during somatostatin, further underscoring the conclusion that hypoinsulinemia reduced protein synthetic rate.

The main mediator for hypermetabolism in severe trauma and sepsis has been claimed to be catecholamines (98). However, recent studies have failed to demonstrate a decrease in metabolic rate in burn patients with adrenergic blockade (99). On the other hand, recent evidence suggest elevated cortisol and particularly glucagon are playing a role (100). Thus a decrease in glucagon would be expected to reduce metabolic rate since somatostatin is not known to have any effect on catecholamines and cortisol secretion (101). The important role of basal insulin concentration on restraining basal metabolic

rate is established by the significant elevation of REE during the somatostatin period as compared to that in the basal period. This finding in septic patients is consistent with the observation that tight control of glucose concentration with insulin in diabetic patients is often clinically associated with decreased energy expenditure and weight gain. Not only did basal insulin restrain overall REE in trauma and septic patients it also minimized the extent of fat oxidation, as indicated by the 40% increase in the rate of fat oxidation during somatostatin. Since insulin is a potent inhibitor of lipolysis (102), it is reasonable to presume that this was the mechanism responsible for the decrease in fat oxidation.

The rapid fall of plasma glucose level during somatostatin infusion was dramatic. Although glucagon was decreased by 64%, it was still more than four times that of the normal value. The persistently high glucagon level, coupled with very low insulin level would normally have been expected to increase plasma glucose production, and thus concentration, as we reported in non-septic burn patients (86). One possible explanation for the hypoglycemia is that compared to burn patients these septic patients relied, to a greater extent, on a high glucagon level for maintenance of hepatic glucose production. When plasma glucagon level became less high due to somatostatin infusion the liver failed to respond to that level of glucagon, hence a decreased glucose production rate resulting in hypoglycemia. This interpretation is consistent with the observation that in experimental sepsis, the gluconeogenic capacity of the isolated liver studied in vitro is significantly impaired, even when taken from animals at a time when glucose production is elevated in vivo (103). Reduction of glucagon with basal replacement of portal insulin in septic dogs

causes severe hypoglycemia (104), further supporting the notion that a markedly elevated concentration of glucagon is necessary to sustain euglycemia in sepsis.

In conclusion, the basal insulin level plays an important role in the regulation of protein and energy metabolism in septic patients. Basal insulin restrains net protein catabolism by inhibiting amino acid oxidation and stimulating protein synthesis. It also prevents a further increase in an already elevated resting energy expenditure and restrains an already increased rate fat oxidation.

Publications

The following publications have resulted from the work sponsored by this three-year contract:

1. Shangraw, R.E., C.A. Stuart, M.J. Prince, E.J. Peters and R.R. Wolfe. Insulin responsiveness of protein metabolism in vivo following bedrest in humans. Am. J. Physiol. 255(Endocrinol. Metab. 18):E548-E558, 1988.

2. Shangraw, R.E., F. Jahoor, H. Miyoshi, W.A. Neff, C.A. Stuart, D.N. Herndon, and R.R. Wolfe. Differentiation between septic and postburn insulin resistance. Metabolism. 38:983-989, 1989.

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TABLE 1. *Glucose and hormone concentrations before and during bedrest*

	Basal Concentrations						Glucose Tolerance Test		Daily Urinary Excretion		
	Blood glucose, mg/dl	Plasma glucagon, pg/ml	Plasma insulin, μU/ml	Growth hormone, ng/ml	Somatomedin C, U/ml	Plasma cortisol, μg/dl	Glucose area, $mg \cdot h^{-1} \cdot dl^{-1}$	Insulin area, $\mu U \cdot h^{-1} \cdot ml^{-1}$	Cortisol, μg/24 h	Metanephrine, mg/24 h	Normetanephrine, mg/24 h
Control	74±3	109±6	6.7±0.6	0.73±0.11	1.28±0.19	15±2	305±27	108±22	44±4	225±22	288±27
Bedrest	74±3	128±7*	9.5±1.6*	0.53±0.06	1.41±0.19	19±1	355±27*	155±32*	51±4*	229±11	245±17*

Values are means ± SE for 6 subjects. * $P < 0.05$ compared with control.

TABLE 2. *Magnetic resonance imaging of back and lower extremity muscle and fat volumes*

Subject No.	Base Line, cm^3		Bedrest, cm^3	
	Muscle	Fat	Muscle	Fat
Calves				
2	1,606	638	1,607	678
3	1,550	504	1,524	513
4	1,536	233	1,534	253
5	1,751	190	1,731	185
6	1,585	512	1,559	485
Means ± SE	1,606±38	415±87	1,591±38 ($P = 0.03$)	423±90 (NS)
Thighs				
2	4,188	1,586	4,159	1,728
3	3,424	1,197	3,369	1,223
4	4,379	605	4,808	602
5	5,468	404	5,371	488
6	3,572	970	3,495	960
Means ± SE	4,306±388	952±210	4,240±382 ($P = 0.002$)	1,000±223 (NS)
Paraspinal muscles				
2	318		299	
3	290		263	
4	319		310	
5	366		354	
6	263		263	
Means ± SE	311±17		298±17 ($P = 0.02$)	

Probabilities were calculated using paired *t* test comparing bedrest to base-line values.

TABLE 3. *Effect of bedrest on nitrogen balance and 3-methyl-L-histidine excretion in humans*

	Base Line			Clamp	Bedrest						Clamp
Hospital Day	2	3	4	5	6	7	8	9	10	11	12
Daily N balance, g/day	1.1±0.9	1.8±0.5	1.3±0.9		1.1±0.6	0.5±0.2	0.2±0.4	0.2±0.2	−0.2±0.2*	0.6±0.6	
Net balance compared with base line, g/day	0	0	0		−0.3±0.6	−0.9±0.2*	−1.2±0.4*	−1.2±0.2*	−1.6±0.2*	−0.8±0.6*	
3-MH excretion, μmol/day	269±27	258±24	291±23		347±37	301±6	304±4	296±14	314±19	301±22	
Cr excretion, mg/day	1,325±205	1,431±196	1,573±158		1,623±153	1,522±101	1,427±167	1,346±139	1,495±189	1,642±127	
3-MH excretion, μmol/g Cr	212±14	186±11	189±14		215±14	202±13	229±29	236±35	226±29	184±8	

Values are means ± SE of 5 subjects. 3-MH, 3-methyl-L-histidine; Cr, creatinine. * $P < 0.04$ compared with control.

TABLE 4. *Effect of insulin infusion on ^{13}C enrichment of plasma leucine*

Subject No.	Insulin Infusion Rate, $mU \cdot m^{-2} \cdot min^{-1}$				
	0	15	40	120	240
Control					
1	7.45	8.45	9.43	10.92	11.56
2	7.15	7.51	8.67	9.65	10.30
3	6.61	7.55	8.59	9.25	8.09
4	6.12	6.48	7.52	7.71	8.00
5	6.64	7.02	7.92	8.21	8.35
6	6.80	7.51	8.27	8.98	7.82
Means	6.80	7.42	8.40	9.12	9.02
±SE	0.19	0.27	0.27	0.46	0.63
Bedrest					
1	6.71	7.52	8.13	10.13	9.48
2	6.96	7.76	8.87	9.69	9.96
3	6.19	6.91	7.71	8.61	8.58
4	6.14	7.09	8.00	9.22	8.75
5	5.35	6.31	7.98	8.34	8.22
6	6.81	7.70	8.92	9.26	9.85
Means	6.36	7.22	8.27	9.21	9.14
±SE	0.24	0.23	0.21	0.27	0.29

Values are in units of atom percent excess enrichment.

TABLE 5. *Effect of insulin infusion on ^{13}C enrichment of plasma α-ketoisocaproic acid*

Subject No.	Insulin Infusion Rate, $mU \cdot m^{-2} \cdot min^{-1}$				
	0	15	40	120	240
Control					
1	5.18	5.41	6.41	7.75	8.05
2	4.30	4.59	5.50	6.27	6.82
3	4.14	4.57	5.65	6.44	6.43
4	3.14	4.43	5.64	6.07	5.84
5	3.82	4.58	5.77	5.87	6.25
6	3.94	4.71	6.50	6.96	7.33
Mean	4.09	4.72	5.91	6.56	6.79
±SE	0.27	0.14	0.18	0.28	0.33
Bedrest					
1	4.96	5.51	6.42	7.34	7.72
2	4.72	5.01	6.17	6.99	5.77
3	3.90	4.69	5.46	6.13	6.35
5	3.92	4.66	6.12	6.68	7.18
6	4.10	5.10	6.40	7.16	7.64
Mean	4.32	4.99	6.11	6.86	6.93
±SE	0.22	0.16	0.17	0.21	0.38

Values are in units of atom percent excess enrichment.

TABLE 6. *Leucine turnover determined by double-labeled infusions*

Experiment	Infusion	Duration, h	Infusion Rate, μmol/kg·min	Atom Percent Excess	Appearance Rate Leucine, μmol/kg·min
1	[1-^{13}C]leucine	2	0.12	6.67±0.68	1.64
	5,5,5-d_3-leucine	2	0.04	2.38±0.27	1.52
2	[1-^{13}C]leucine	2	0.12	6.25±0.04	1.76
	5,5,5-d_3-leucine	2	0.04	2.19±0.02	1.67
3	[1-^{13}C]leucine	10	0.12	8.68±0.09	1.22
	5,5,5-d_3-leucine	2	0.04	2.91±0.06	1.21
4	[1-^{13}C]leucine	10	0.12	8.59±0.22	1.24
	5,5,5-d_3-leucine	2	0.04	2.84±0.06	1.25

TABLE 7. *Effect of insulin on whole body protein breakdown before and during bedrest*

	Insulin Infusion, $mU \cdot m^{-2} \cdot min^{-1}$				
	0	15	40	120	240
Control	284±17	246±8*	189±5*	173±7*	167±8*
Bedrest	270±13	230±7*	187±6*	163±6*	160±5*

Values are means ± SE of 6 subjects in mg protein·kg body $wt^{-1} \cdot h^{-1}$. * $P < 0.01$ compared with basal period. There was no difference between control and bedrest at any insulin infusion rate.

TABLE 8. *Effect of insulin on plasma amino acid levels before and during bedrest*

Amino Acid	Insulin Infusion, $mU \cdot m^{-2} \cdot min^{-1}$				
	0	15	40	120	240
Asp					
Control	15±2	13±1	11±1	11±1	10±1
Bedrest	14±1	15±2	11±1	12±1	11±1
Thr					
Control	111±11	99±10	78±10*	67±7*	57±9*
Bedrest	116±14	112±12	81±14*	65±7*	62±8*
Ser					
Control	94±10	86±10	69±11	62±5*	53±9*
Bedrest	93±11	95±10	77±12	61±7*	60±9*
Glu + Gln					
Control	594±23	554±41	469±27*	388±29*	339±29*
Bedrest	584±34	518±29	436±35*	328±17*	320±20*
Pro					
Control	167±23	148±18	126±13	118±10	98±10*
Bedrest	174±18	168±16	139±16	105±9*	103±13*
Gly					
Control	171±21	177±20	151±26	146±19	132±19
Bedrest	190±22	208±20	192±30	154±16*	154±23*
Ala					
Control	248±16	247±26	220±26	195±26	176±26
Bedrest	305±42	291±27	231±29	187±15*	185±24*
Val					
Control	156±14	133±9	97±7	80±6*	60±6*
Bedrest	148±9	143±2	105±6*	69±2*	62±5*
Met					
Control	17±1	15±1	9±1*	6±1*	5±1*
Bedrest	20±1	18±1	12±2*	6±1*	5±1*
Ile					
Control	40±3	28±3	13±1*	6±1*	5±1*
Bedrest	41±3	37±3	18±2*	10±1*	6±1*
Leu					
Control	95±6	80±5	54±4*	43±3*	38±4*
Bedrest	91±4	86±6	61±2*	47±4*	39±3*
Tyr					
Control	38±2	34±2	23±2*	20±1*	15±1*
Bedrest	39±3	36±3	25±2*	16±1*	15±1*
Phe					
Control	38±2	35±2	27±3*	24±3*	20±2*
Bedrest	41±2	38±2	27±2*	20±1*	17±2*
Lys					
Control	130±13	139±10	109±16	94±10*	80±12*
Bedrest	136±16	144±15	125±16	93±10*	86±11*
His					
Control	65±10	71±12	59±11	56±8	52±10
Bedrest	69±10	72±7	70±9	58±8	59±7
Arg					
Control	72±14	75±17	58±13	51±7	45±14*
Bedrest	69±14	77±16	55±11	45±8	44±12

Values are means ± SE of 6 subjects in μmol/l. * $P < 0.03$ compared with basal period. There was no difference between control and bedrest at any insulin infusion rate. Asp, aspartic acid; Thr, threonine; Ser, serine; Glu, glutamic acid; Gln, glutamine; Pro, proline; Gly, glycine; Ala, alanine; Val, valine; Met, methionine; Ile, isoleucine; Leu, leucine; Tyr, tyrosine; Phe, phenylalanine; Lys, lysine; His, histidine; Arg, arginine.

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Table 1. Diagnosis, Hemodynamics, and Severity of Septic Patients

Subjects (age/sex)	Diagnosis	Cardiac Index ($L/m^2 \cdot mm\ Hg$)	SVRI ($dyne/m^2 \cdot mm\ Hg$)	Sepsis Score	APACHE II Score	Outcome
46/M	Perforated appendicitis	7.18	1,022	14	8	Survived
33/M	Small bowel infarction	5.34	868	18	13	Survived
36/M	90% Body surface flame burn	5.87	841	17	16	Died
28/M	60% Body surface flame burn	8.80	780	12	16	Died
41/M	Pneumonia	5.54	577	20	19	Died
35/M	Pancreatic abscess	6.80	823	16	13	Survived
44/F	Pneumonia	6.16	636	22	25	Died
43/M	43% Body surface flame burn, pancreatitis	5.94	859	16	12	Survived

Abbreviation: SVRI, systemic vascular resistance index.

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Table 2. Wound Size, Extent, and Severity of Nonseptic Burn Patients

Subject (age)	Burn Wound (%TBSA, mechanism)	Full Burn Thickness (%TBSA)	Inhalation Injury (per bronchoscopy)	Injury Severity Score	Outcome
31	47, flame	4	no	38	Survived
20	51, flame	12	no	29	Survived
16	75, flame	33	yes	37	Survived
50	48, flame	5	no	25	Survived
42	45, flame	15	no	26	Survived
28	80, flame	70	no	41	Died
21	83, flame	80	yes	40	Survived
18	73, flame	62	yes	36	Survived

Abbreviation: TBSA, total body surface area.

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Table 1. Basal Metabolic Characteristics

	Group		
	Bed-Rested	Septic	Burned
Age (yr)	23 ± 1	38 ± 2*	28 ± 4
Weight (kg)	66 ± 6	82 ± 7	72 ± 4
Body mass index	22.6 ± 1.3	28.2 ± 1.6*†	23.6 ± 1.3
Ideal body weight (%)	102 ± 6	128 ± 7*†	107 ± 6
RQ	0.83 ± 0.01	0.77 ± 0.01*	0.79 ± 0.02
REE (kcal/hr)	62 ± 1	123 ± 15	115 ± 6
REE (% predicted)	90 ± 3	148 ± 9*	161 ± 7*
Plasma Glucose (mg/dL)	80 ± 3	108 ± 7*	108 ± 6*
Plasma Insulin (uU/ml)	10 ± 1	18 ± 6	9 ± 1
Hepatic Glucose Production (mg/kg · min)	1.86 ± 0.06	3.03 ± 0.15*†	3.97 ± 0.18*

NOTE. Body mass index is defined as weight (kg) ÷ height^2 (m). Ideal body weight calculated from medium-frame ideal body weight estimates. REE calculated as $[(3.94 \times \dot{V}o_2\ (L/min)] + (1.1 \times \dot{V}co_2\ (L/min)] \times 1420$. Plasma glucose measured from normal volunteers not restricted to bed. Bed rest does not cause significant changes in hepatic glucose production.[10]

*$P < .02$ *v* bed-rested.

†$P < 0.01$ *v* burned.

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Table 2. Glucose Uptake During Euglycemic Clamp Technique

Subjects	Plasma Insulin (μU/mL)	Glucose Utilization (mg/kg · min)	Insulin Infusion (mU/m^2/min)
A. Higher Insulin Dose			
Bed-rested (n = 6)	736 ± 31	13.73 ± 1.10	240
Septic (n = 5)	1,298 ± 347	7.11 ± 0.44*†	400
Burned (n = 6)	1,213 ± 229	12.40 ± 0.72	500
B. Lower Insulin Dose			
Bed-rested (n = 6)	220 ± 18	11.41 ± 0.93	120
Septic (n = 3)	329 ± 39	5.79 ± 0.86*†	240
Burned (n = 3)	329 ± 85	11.43 ± 1.44	240

*$P < .001$ *v* bed-rested.

†$P < .001$ *v* nonseptic burned.

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Table 3. Metabolic Response to Hyperinsulinemic-Euglycemic Clamp Technique

Metabolic Factor	Burned Patients		Septic Patients	
	Basal	Clamp	Basal	Clamp
Oxygen consumption (mL/min)	399 ± 24	449 ± 31*	420 ± 41	432 ± 36
CO_2 production (mL/min)	312 ± 16	401 ± 29*	321 ± 30	358 ± 32
RQ	0.79 ± 0.02	0.90 ± 0.03†	0.77 ± 0.03	0.82 ± 0.04*
Carbohydrate oxidation (mg/kg · min)	1.59 ± 0.3	4.77 ± 0.7*	1.20 ± 0.3	3.28 ± 0.6*
Glucose uptake oxidized (%)	38 ± 1.7	42 ± 6.4	38 ± 2.3	51 ± 5.8*

*Significantly different from basal value ($P < .05$).

TABLE 14 - CHARACTERISTICS OF BURN PATIENTS

Patient	Age Yr.	Sex	Weight (kg)	% BSA Burn Total/3	Postburn Day Studied	Protocol #	Heart Rate beats/min	Blood Pressure mm Hg	Temperature C
A*	31	M	64	47/4	14;17	1;2	130;116	128;126	38.7;38
B*	20	M	71	51/12	13;16	1;2	104;95	132;135	38.6;38.6
C*	16	M	65	75/33	8;11	1;2	130;122	130;145	38.1;37.7
D*	50	M	83	48/5	12;15	1;2	114;131	110;100	38;39
E*	42	M	86	45/15	5;8	1;2	95;104	141;108	37.7;38.6
F	21	M	61	83/80	13	1	125	120	37.3
G	18	F	45	73/60	4	1	122	133	37.8
H	28	M	90	81/60	16	1	136	132	37.7

M = male, F = female, BSA = body surface area.

*Patient studied in protocols 1 and 2.

Values after semicolon are for protocol 2.

TABLE 15 - CHARACTERISTICS OF SEPTIC PATIENTS

Patient	Age Yr.	Sex	Weight (kg)	Diagnosis	Sepsis Score	Cardiac Index $l/min^{-1}m^{-2}$	Heart Rate beats/min	Blood Pressure mm Hg	Temperature C
I	45	M	85	Perforated Appendicitis	14	7.2	102	160	37.8
J*	33	M	125	Small Bowel Infarcation	18	5.3;5.5	108;112	116;114	37.6;38
K	35	M	62	90% BSA Burn	17	5.9	129	129	38.3
L	28	M	99	60% BSA Burn	12	8.8	114	126	37.3
M*	41	M	90	Pneumonia	20	5.5;5.4	128;96	121;100	37.9;37.3
N	35	M	71	Pancreatic Abscess	16	6.8	123	169	37.7
O	44	F	57	Pneumonia	22	6.2	118	100	38.9
P*	43	M	81	43% BSA burn; Pancreatitis	16	5.9;5.9	101;105	132;130	39.2;38.5

M = male; F = female; BSA = body surface area.

* Patient studied in protocols 1 and 2.

Values after semicolon are for protocol 2.

TABLE 16

RESPONSE OF LEUCINE AND UREA KINETICS TO HYPERINSULINEMIC EUGLYCEMIC CLAMP

Subject Group	Basal	Clamp
	Leucine Ra (umol kg^{-1} min^{-1})	
Control * (n=6)	2.78 ± 0.16	1.64 ± 0.08^{+}
Burn (n = 8)	5.15 ± 0.24	3.39 ± 0.14^{+}
Sepsis (n = 8)	4.08 ± 0.22	2.99 ± 0.19^{++}
	Leucine Oxidation	
Control *	0.61 ± 0.07	0.32 ± 0.01^{+}
Burn	1.60 ± 0.14	1.00 ± 0.11^{+}
Sepsis	1.25 ± 0.15	1.06 ± 0.11^{++}
	Urea Ra	
Control (n = 14)**	4.20 ± 0.37	---
Burn	8.19 ± 0.92	7.01 ± 0.66^{++}
Sepsis	6.23 ± 1.15	5.49 ± 0.99^{++}

Values are means ± SEM.

$^{+}$, $^{++}$, Significantly different from basal value ($p < 0.01$, $p < 0.05$) respectively.

* From Reference No. 24.

** From Reference No. 16.

TABLE 17

RESPONSE OF PLASMA AMINO ACID CONCENTRATIONS TO A HYPERINSULINEMIC EUGLYCEMIC CLAMP IN BURN AND SEPTIC PATIENTS (Values are Mean + SEM)

Amino Acid	Burn		p*	Sepsis		p
	Basal	Clamp		Basal	Clamp	
	uM			uM		
Aspartate	24±2	16±1	< 0.01	26±3	21±3	< 0.01
Glutamate plus Glutamine	296±32	202±20	< 0.01	410±41	262±33	< 0.01
Alanine	193±21	130±15	< 0.01	280±42	205±29	< 0.01
Glycine	141±10	110±4	< 0.01	177±19	144±16	< 0.01
Serine	79±7	43±4	< 0.01	76±4	54±5	< 0.01
Proline	144±15	85±8	< 0.01	227±49	149±29	< 0.01
Threonine	79±7	38±4	< 0.01	89±8	56±6	< 0.01
Methionine	25±2	10±1	< 0.01	35±6	17±3	< 0.01
Lysine	148±6	96±4	< 0.01	172±19	124±13	< 0.01
Histidine	56±4	45±3	< 0.05	96±17	78±14	< 0.01
Valine	191±21	75±9	< 0.01	179±23	106±22	< 0.01
Isoleucine	60±9	21±3	< 0.01	58±4	21±4	< 0.01
Leucine	127±13	60±6	< 0.01	144±19	78±14	< 0.01
Tyrosine	61±5	29±2	< 0.01	70±5	44±4	< 0.01
Phenylalanine	78±4	54±3	< 0.01	115±5	86±6	< 0.01
Arginine	69±6	45±6	< 0.01	79±7	54±6	< 0.01

Values are mean ± SE.

*Significance of difference from basal.

TABLE 18

EFFECT OF DICHLOROACETATE ON THE METABOLIC RESPONSE TO THE HYPERINSULINEMIC EUGLYCEMIC CLAMP (PROTOCOL 2)

(Values are Mean ± SEM)

Metabolic Parameter	Basal	Clamp	Basal	Clamp + DCA
VO_2 (ml/min)	415±29	439±17	415±35	421±24
VCO_2 (ml/min)	331±17	407±19$^+$	322±24	408±25$^+$
RQ*	0.80±0.02	0.92±0.02$^+$	0.78±0.01	0.97±0.02$^+$
REE* (kcal/m^2/d)	1388±103	1487±89	1412±106	1534±55

*RQ is Respiratory Quotient.

REE is resting energy expenditure.

$^+$Significantly different from basal value ($p < 0.01$).

TABLE 19

EFFECT OF DICHLOROACETATE ON PLASMA LACTATE, ALANINE AND UREA CONCENTRATIONS DURING HYPERINSULINEMIC EUGLYCEMIC CLAMP
(Values are mean ± SEM)

Substrate	Basal	Clamp	Basal	Clamp + DCA
Lactate (umol/ml)	2.31±0.79	3.09±0.91^{+}	2.03±0.33	0.91±0.08^{+}
Alanine (umol/ml)	250±36	171±23^{+}	230±26	71±6^{+}
Urea (umol/ml)	7.69±1.52	6.02±1.05^{+}	6.32±0.80	4.97±0.68^{+}

$^{+}$ Significantly different from basal value, ($p < 0.05$).

TABLE 20

EFFECT OF DICHLOROACETATE ON RESPONSE OF LEUCINE AND UREA KINETICS TO HYPERINSULINEMIC EUGLYCEMIC CLAMP (PROTOCOL 2)

	Basal	Clamp	Basal	Clamp + DCA
	(μmol kg^{-1} min^{-1})			
Leucine Ra	4.59±0.34	3.05±0.19^{+}	4.55±0.34	2.54±0.27^{+}
Δ Leucine Ra		1.54±0.18		2.01±0.24
Leucine Oxidation	1.58±0.16	0.94±0.11^{+}	1.40±0.13	0.79±0.03^{+}
Δ Leucine Oxidation		0.64±0.13		0.62±0.09
Urea Ra	7.86±1.09	6.67±0.8^{++}	6.53±0.61	5.78±0.05^{++}
Δ Urea Ra		1.19±0.27		0.75±0.12

Values are mean ± SE for 8 patients (5 burns, 3 septic).

$^{+}$, $^{++}$, Significantly different from basal value ($p < 0.01$, $p < 0.05$), respectively.

Δ Change from basal due to the clamp (Basal-Clamp) and due to clamp + DCA (Basal - Clamp + DCA).

TABLE 21

EFFECT OF DICHLOROACETATE ON THE RESPONSE OF PLASMA AMINO ACID CONCENTRATIONS TO THE HYPERINSULINEMIC EUGLYCEMIC (PROTOCOL 2)

Values are Mean ± SEM

Amino Acid	Basal	Clamp	p	Basal	Clamp + DCA	p
	uM			uM		
Aspartate	26±1	18±2	0.01	22±2	18±2	N.S.
Glutamate + Glutamine	370±30	216±21	< 0.01	318±44	210±10	< 0.01
Alanine	250±36	171±23	< 0.01	231±26	71±6	< 0.01
Glycine	161±17	125±13	< 0.01	152±12	130±5	N.S.
Serine	84±5	47±3	< 0.01	75±6	42±3	< 0.01
Proline	193±45	115±28	< 0.01	170±16	76±9	< 0.01
Threonine	91±7	46±6	< 0.01	87±7	45±6	< 0.01
Methionine	31±5	14±3	< 0.01	24±1	9±1	< 0.01
Lysine	158±10	105±9	< 0.01	162±16	108±7	< 0.01
Histidine	76±16	60±13	< 0.01	60±6	50±9	N.S.
Valine	205±22	88±12	< 0.01	187±27	85±13	< 0.01
Isoleucine	66±7	20±2	< 0.01	60±6	22±3	< 0.01
Leucine	149±14	71±10	< 0.01	131±16	60±6	< 0.01
Tyrosine	71±3	35±4	< 0.01	69±7	35±5	< 0.01
Phenylalanine	92±6	62±7	< 0.01	83±9	53±5	< 0.01
Arginine	78±5	55±3	< 0.01	76±6	50±4	< 0.05

*Significance of difference from basal values (paired t-test).

TABLE 22 - CHARACTERISTICS OF SEPTIC PATIENTS

Case	Age Yr.	Sex	Primary Diagnosis	Sepsis Diagnosis	Sepsis Score	Heart Rate Beats/min	Respir. Rate times/min	Blood Pressure mmHg	Body Core Temperature °C
A	22	F	55% TBSA Burn (3°,0%)	Wound Sepsis	9	112	22	120/70	38.2
B	23	M	50%TBSA Burn (3°,18%)	Septicemia	18	120	28	105/50	38.1
C	58	M	60% TBSA Burn (3°,0%)	Bacteremia	12	118	20	110/60	38.8
D	48	M	50% TBSA Burn (3°,2%)	Septicemia	22	118	18	125/64	38.0
E	48	M	Acute Pancreatitis	Peritonitis	19	100	30	135/70	38.6
F	52	F	Incarcerated Ventral Hernia	ARDS Pneumonia	16	80	14	120/70	36.4
G	41	M	Multiple Trauma	Pneumonia	8	110	30	130/80	37.6
H	28	M	Gun Shot Wound	Peritonitis	18	142	32	138/76	38.2

TABLE 23 - RESPONSE OF LEUCINE KINETICS TO SOMATOSTATIN IN SEPTIC PATIENTS

		Rate of Appearance	Rate of Oxidation	Appearance minus Oxidation
All Patients	Basal	3.90±0.21+	0.95±0.08	2.95±0.18
(n=8)	Somat	3.85±0.18	1.18±0.14*	2.67±0.17*
Septic Burn	Basal	4.03±0.05	1.04±0.13	2.99±0.24
(n=4)	Somat	4.11±0.05	1.32±0.17	2.79±0.19
Non-Burn	Basal	3.76±0.31	0.85±0.09	2.91±0.31
Sepsis (n=4)	Somat	3.59±0.31	1.04±0.20	2.55±0.29

Note: + values are mean±SEM. * $p < 0.01$ compared to basal values. Basal and Somat denote Basal period and Somatostatin period.

TABLE 24 - RESPONSE OF SUBSTRATE OXIDATION AND RESTING ENERGY EXPENDITURE TO SOMATOSTATIN INFUSION IN SEPTIC PATIENTS

	Basal	Somat
REE (Kcal/M^2.day)	1310±100+	1505±128*
CO_2 Production (L/min)	0.282±0.027	0.307±0.027*
O_2 Consumption (L/min)	0.365±0.033	0.423±0.041*
CHO-Oxidation (mg/kg.min)	1.51±0.49	1.31±0.49
Fat-Oxidation (mg/kg.min)	1.72±0.24	2.41±0.41

Note: + values are mean±SEM.*p < 0.05 compared with Basal values. Somat denotes somatostatin period; CHO, Carbohydrate; REE, Resting energy expenditure.

TABLE 25 - RESPONSE OF PLASMA AMINO ACID PROFILE

	Basal Period	Somat Period
Aspartic Acid	14 ± 2	15 ± 4
Threonine	62 ± 10	77 ± 14*
Serine	57 ± 7	71 ± 8*
Glumatic Acid	104 ± 38	121 ± 52
Glutamine	141 ± 52	201 ± 64*
Proline	135 ± 16	158 ± 13
Glycine	132 ± 20	158 ± 26
Valine	166 ± 18	196 ± 25
Alanine	143 ± 27	175 ± 38
Methionine	29 ± 11	28 ± 9
Isoleucine	52 ± 41	61 ± 16
Leucine	115 ± 31	155 ± 31**
Tyrosine	58 ± 9	64 ± 11
Phenylalanine	94 ± 21	101 ± 23
Lysine	155 ± 11	163 ± 16
Histidine	99 ± 11	88 ± 12
Arginine	72 ± 15	71 ± 11
Total Amino Acids	1629 ± 199	1899 ± 202*
Total Amino-N	2340 ± 278	2657 ± 266

Note : Values are Mean ± SEM. * denotes $p<0.05$; **, $p<0.01$ When compared with basal period values. Somat, Somatostatin.

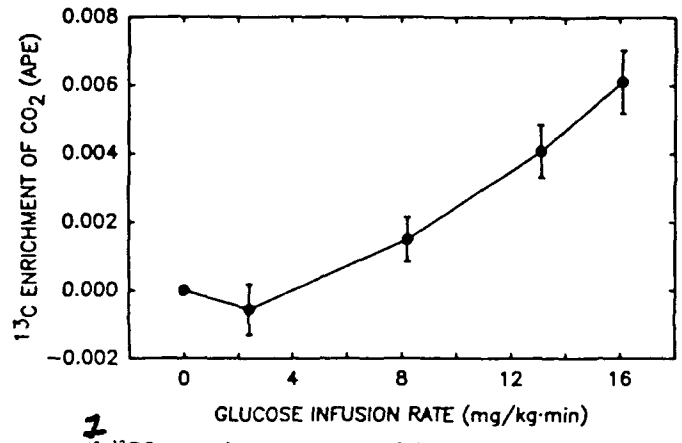


FIG. 1. $^{13}CO_2$ enrichment of expired breath during euglycemic clamp, using "unenriched" glucose infusion. Overnight fasted subjects ($n = 4$) were studied by multidose sequential euglycemic clamp. Insulin was infused at 15, 20, 120, and 240 $mU \cdot m^{-2} \cdot min^{-1}$ for 2-h periods, as described in METHODS. Glucose infusion (15 g/dl) was varied to maintain whole blood glucose at 85 ± 3 mg/dl. Units are atom percent excess compared with enrichment before glucose or insulin infusion. Expired breath taken over final 30 min of each infusion period was immediately bubbled through 10 ml of 0.1 N NaOH to trap CO_2 as $NaHCO_3$. Enrichment of $^{13}CO_2$ was determined by isotope ratio mass spectrometry (IRMS, Nuclide, State College, PA). Data are expressed as means ± SE.

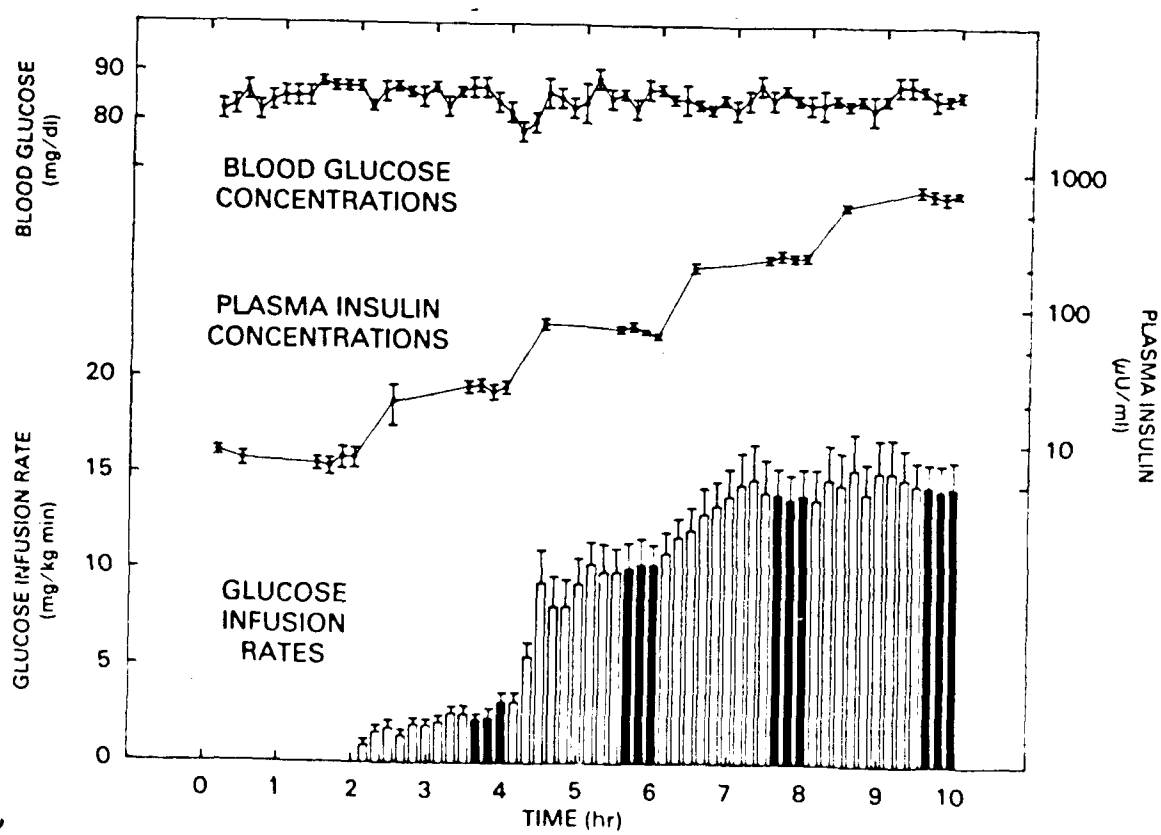


FIG. 2. Multidose sequential euglycemic clamp. Shown here are blood glucose, plasma insulin, and glucose infusion data from base-line clamps performed in our 6 subjects. ■, glucose infusion rates for each 10-min segment of last 30 min of each 2-h insulin infusion period.

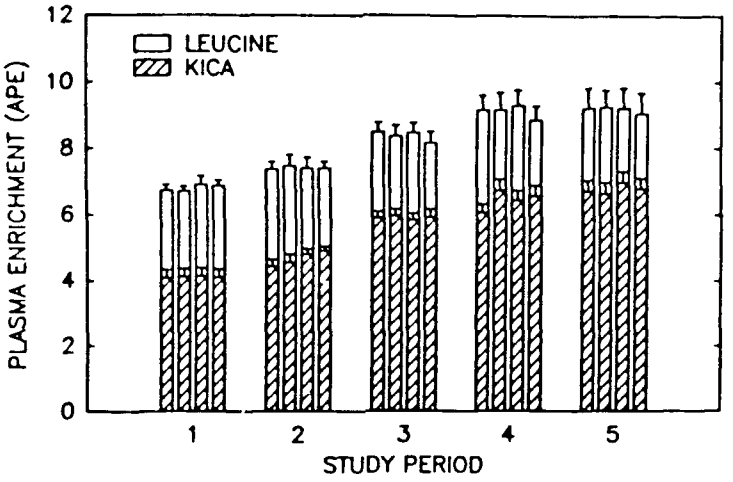


FIG. 3. Effect of insulin infusion on ^{13}C enrichment of plasma leucine and α-ketoisocaproic acid (KICA). [1-^{13}C]leucine was infused at 0.12 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ during a 5-period multidose euglycemic clamp. Data shown here are means ± SE of plasma enrichments of leucine and KICA at 10-min intervals in last 30 min of each 2-h clamp period for 6 normal subjects in their base-line clamp studies.

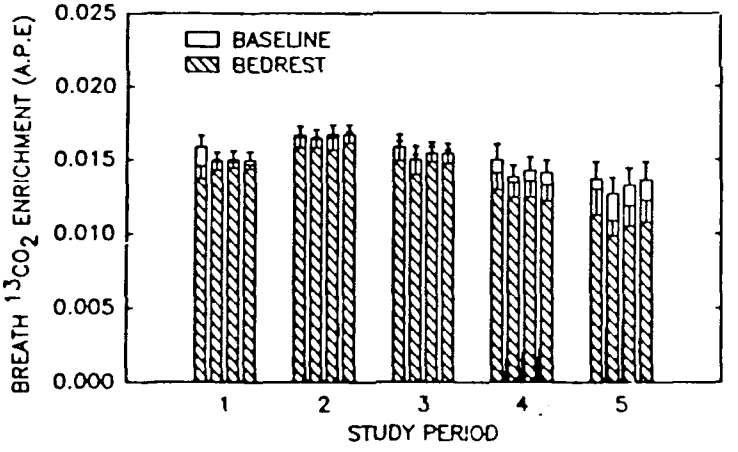


FIG. 4. Effect of insulin infusion during euglycemic clamp studies on ^{13}C enrichment of expired CO_2 in subjects receiving [1-^{13}C]leucine infusions at 0.12 $\mu mol \cdot kg^{-1} \cdot min^{-1}$. Displayed are means ± SE of breath enrichments determined at 10-min intervals in last 30 min of each 2-h clamp period. □, data determined during euglycemic clamp study performed on 7th day of bedrest.

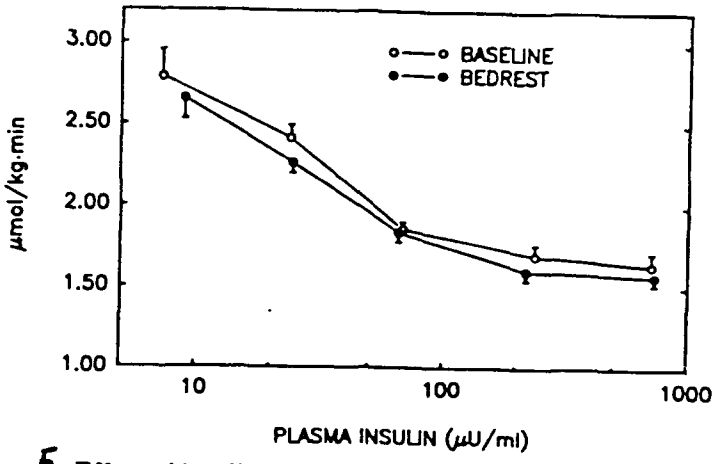


FIG. 5. Effect of insulin on rate of appearance (R_a) of intracellular leucine. Overnight-fasted subjects were infused with insulin at 0, 15, 40, 120, and 240 $mU \cdot m^{-2} \cdot min^{-1}$ during a euglycemic clamp. A primed-constant infusion of [1-^{13}C]leucine (0.12 $\mu moles \cdot kg^{-1} \cdot min^{-1}$) was maintained throughout the study. Subjects maintained their usual physical activity for control period and were subsequently restudied after 7 days of strict bedrest. Values are expressed as means ± SE of 6 subjects. * $P < 0.01$ compared with basal rate within same group. There was no difference between control and bedrested subjects at any insulin infusion rate.

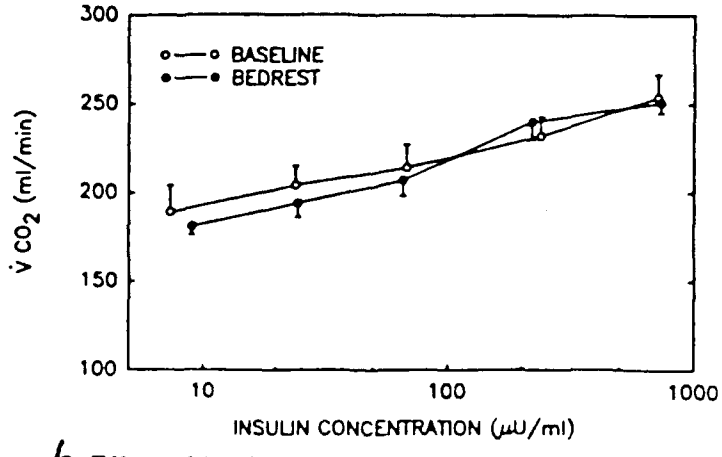


FIG. 6. Effect of insulin infusion on CO_2 production ($\dot{V}CO_2$) in 6 normal subjects before and after 7 days of bedrest. Data displayed are means ± SE for $\dot{V}CO_2$, determined as described in METHODS. $\dot{V}CO_2$ rates determined during euglycemic clamp studies on 7th day of bedrest were not changed compared with data of base-line clamps.

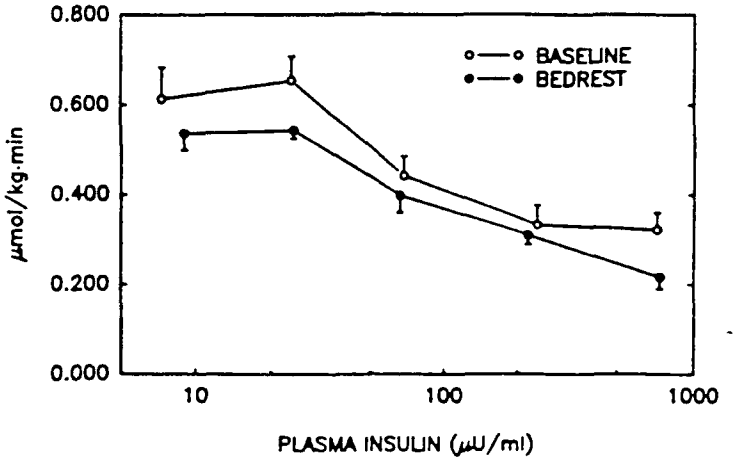


FIG. 7. Effect of insulin on leucine oxidation in vivo in humans. Overnight-fasted subjects were infused with insulin at 0, 15, 40, 120, and 240 $mU \cdot m^{-2} \cdot min^{-1}$ during a euglycemic clamp. A primed-constant infusion of [1-^{13}C]leucine (0.12 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) was maintained throughout study. Subjects maintained their usual physical activity for control period and were subsequently restudied after 7 days of strict bedrest. Values are means ± SE of 6 subjects. * $P < 0.01$ compared with basal rate within same group by ANOVA repeated measures. There was no difference between control and bedrested subjects at any insulin infusion rate.

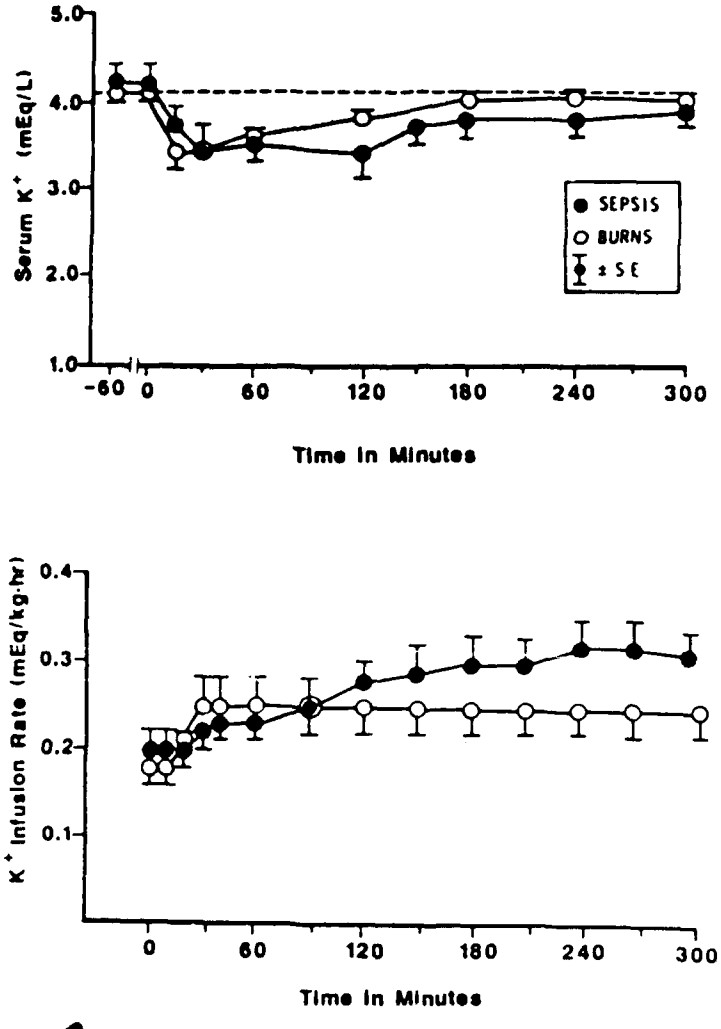


Fig 8 Time course of potassium uptake from the plasma by septic and burned patients during the euglycemic clamp. Postabsorptive subjects received a primed-constant infusion of insulin starting at time zero. The plasma insulin concentrations of septic patients were not different from those of burned patients. Potassium infusion rate, in mEq/kg · h, is expressed as that above basal maintenance infusion rate. Data are averages of five patients ± SE.

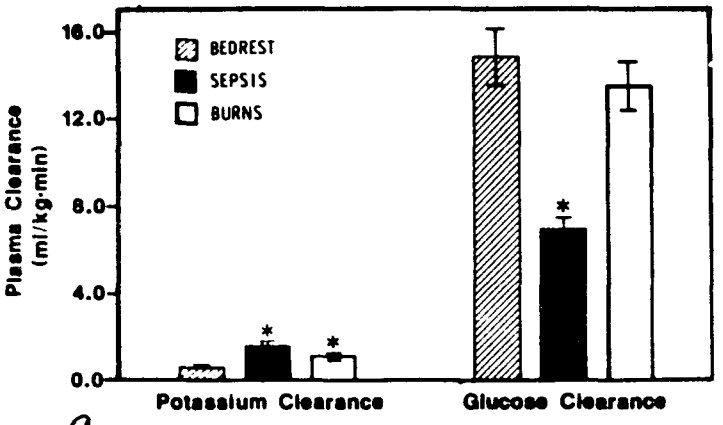
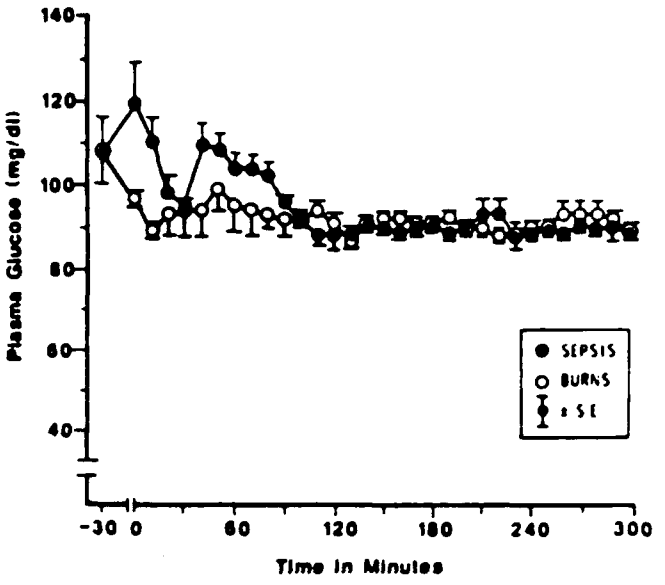


Fig 9 Steady-state clearance of potassium and glucose from the plasma in response to insulin infusion during euglycemic clamp. Postabsorptive septic and burned patients are compared with bed-rested volunteers. Data are averages of five to six subjects ± SE. (*), Significant difference from bedrested controls ($P < .05$).



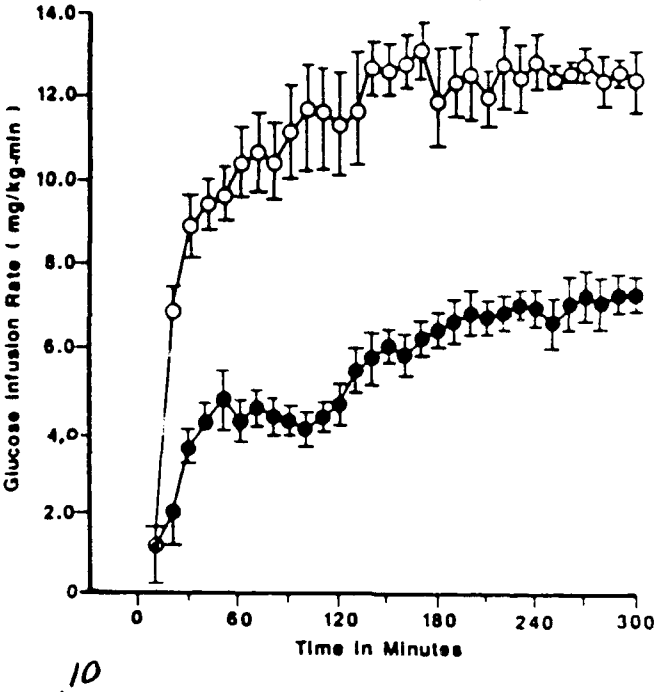


Fig 10 Time course of glucose uptake from the plasma by septic and burned patients during the euglycemic clamp. Postabsorptive subjects received a primed-constant infusion of insulin starting at time zero. The plasma insulin concentrations of septic patients did not differ from those of burned patients. Stable plasma glucose concentration was maintained by variable infusion of aqueous glucose (20 g/dL). Data are averages of five patients ± SE.

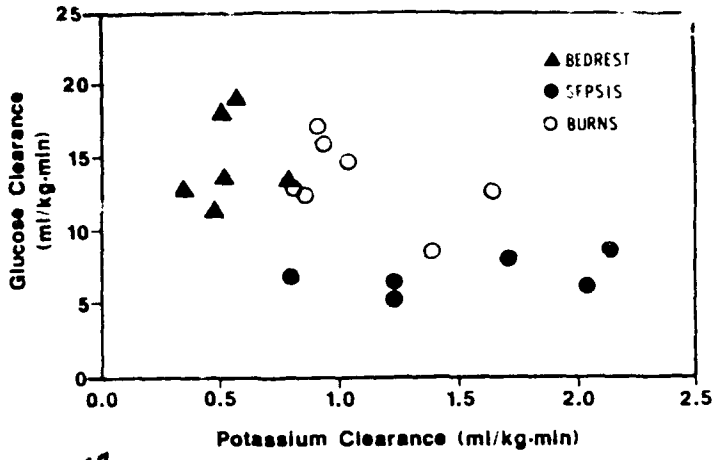


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Fig. Relationship of insulin-stimulated, potassium, and glucose clearances during euglycemic clamp. Postabsorptive septic and burned patients are compared with volunteers restricted to bed for 1 week. Each data point represents an individual subject.

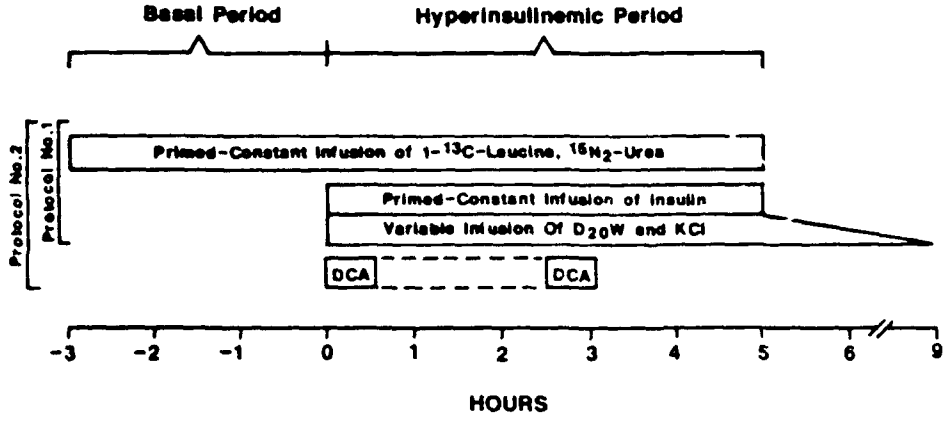

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FIG. 4 Schematic representation of experimental protocols. DCA, dichloroacetate.

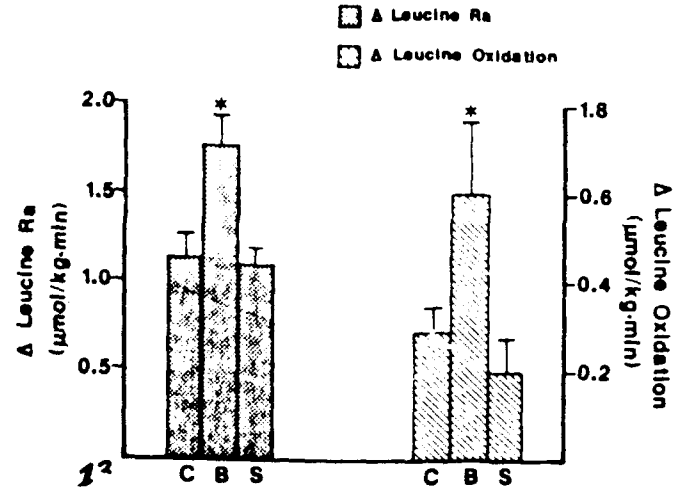

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FIG. Decrease from basal value of leucine rate of appearance (R_a) and oxidation due to hyperinsulinemic clamp in *protocol 1*. C, control subjects; B, burn patients; S, septic patients. Results are means ± SE.
* Significantly different from control value ($P < 0.01$).

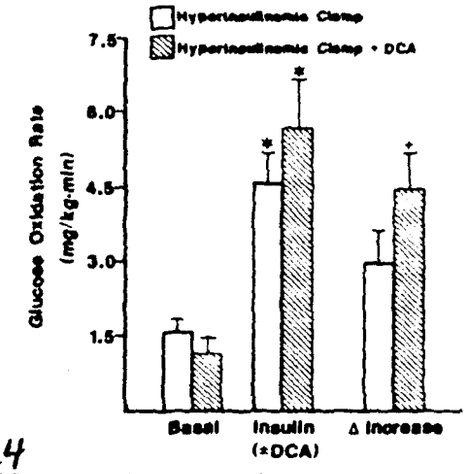

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FIG. 2. Response of glucose oxidation rate to hyperinsulinemic clamp [± dichloroacetate (DCA)] in *protocol 2*. Values are means ± SE. Δ, Increase from basal value. * Significantly different from basal value ($P < 0.01$). + Significantly different from hyperinsulinemic period ($P < 0.05$).

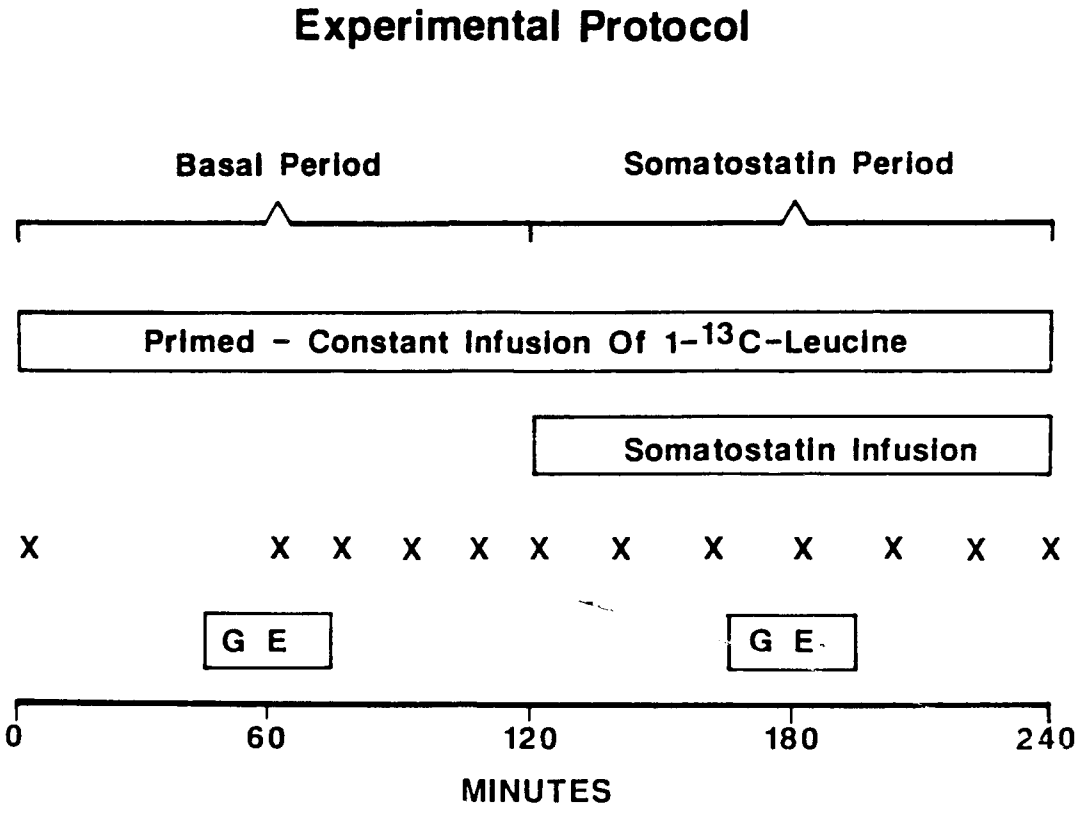

FIGURE 15: A scematic representation of the experimental protcol. X denotes blood and /or expired CO_2 sample; GE , gaseous exchange measurement.

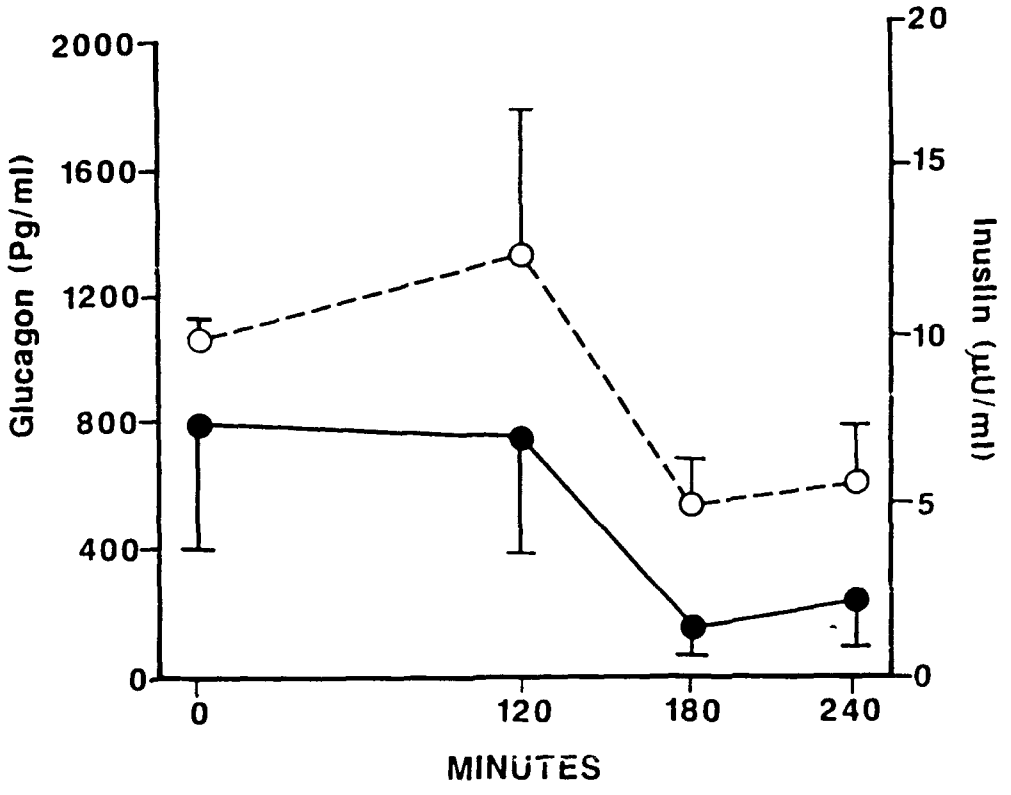

FIGURE 16: Response of plasma glucagon (●———●) and insulin (o- - - o) levels to somastatin infusion.

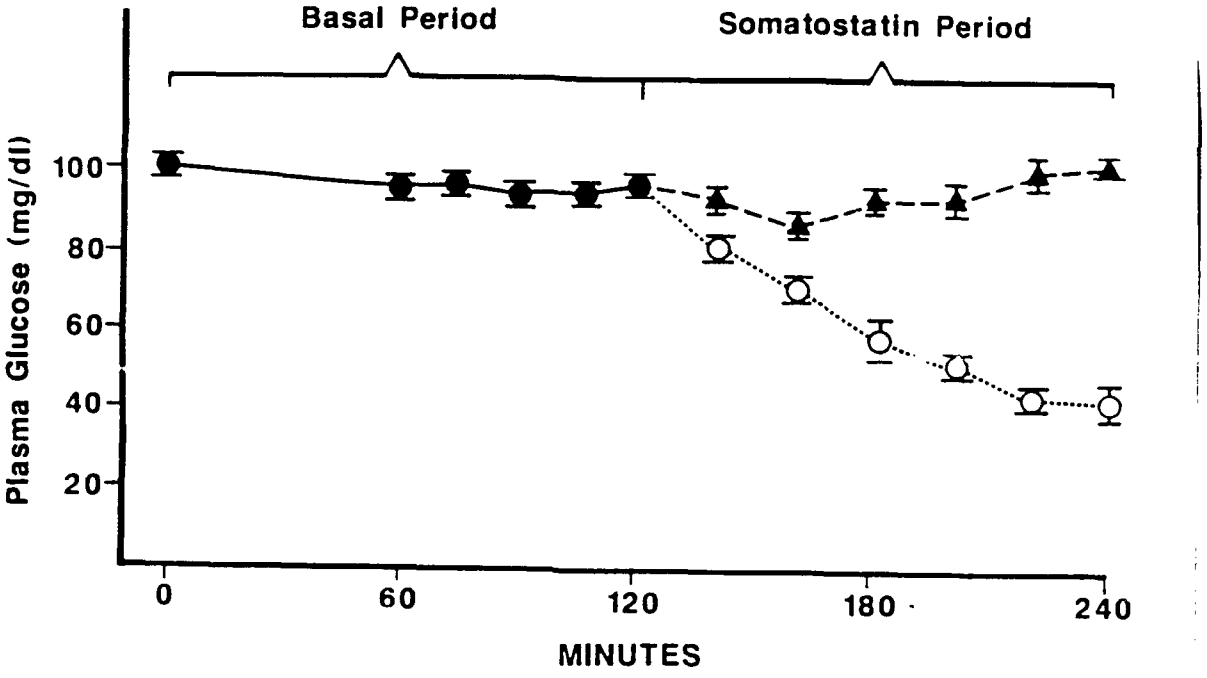

FIGURE 17: Response of plasma glucose concentration from basal period (●——●) to samtostatin period in five patients with glucose replacement (▲- - - -▲) and in three patients without glucose replacement (○·····○).