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Extremity hyperinsulinemia stimulates muscle protein synthesis in severely injured patients

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Gore, Dennis C., Steven E. Wolf, Arthur P. Sanford, David N. Herndon, and Robert R. Wolfe. Extremity hyperinsulinemia stimulates muscle protein synthesis in severely injured patients. *Am J Physiol Endocrinol Metab* 286: E529–E534, 2004. First published December 9, 2003; 10.1152/ajpendo.00258.2003.—Insulin has a well-recognized anabolic effect on muscle protein, yet critically ill, severely injured patients are often considered “resistant” to the action of insulin. The purpose of this study was to assess the *in vivo* effects of hyperinsulinemia on human skeletal muscle in severely injured patients. To accomplish this goal, 14 patients with burns encompassing >40% of their body surface area underwent metabolic evaluation utilizing isotopic dilution of phenylalanine, femoral artery and vein blood sampling, and sequential muscle biopsies of the leg. After baseline metabolic measurements were taken, insulin was infused into the femoral artery at $0.45 \text{ mIU} \cdot \text{min}^{-1} \cdot 100 \text{ ml leg volume}^{-1}$ to create a local hyperinsulinemia but with minimal systemic perturbations. Insulin administration increased femoral venous concentration of insulin ($P < 0.01$) but with only a 4% (insignificant) decrease in the arterial glucose concentration and a 7% (insignificant) decrease in the arterial concentration of phenylalanine. Extremity hyperinsulinemia significantly increased leg blood flow ($P < 0.05$) and the rate of muscle protein synthesis ($P < 0.05$). Neither the rate of muscle protein breakdown nor the rate of transmembrane transport of phenylalanine was significantly altered with extremity hyperinsulinemia. In conclusion, this study demonstrates that insulin directly stimulates muscle protein synthesis in severely injured patients.

insulin resistance; phenylalanine; burns; muscle catabolism; critical illness; hypermetabolic response

INSULIN HAS A WELL-RECOGNIZED ANABOLIC EFFECT ON muscle protein (10, 19). Yet there has been contention as to the mechanism by which insulin mediates this anabolism. For example, several studies have found that insulin promotes an increase in protein synthesis (8, 11), whereas other researchers have evidenced that insulin mediates an inhibition of protein breakdown (12, 17). Insulin also has other physiological actions, such as hypoglycemia and hypoaminoacidemia, effects that may confound a clear understanding of the mechanism by which insulin affects muscle protein (1, 21). To circumvent these compounding variables and discern clearly the isolated effect of insulin on human skeletal muscle protein, Biolo et al. (3) infused insulin into the femoral artery of healthy volunteers and thereby significantly increased insulin availability within the leg (i.e., muscle), yet with minimal systemic perturbations. Using phenylalanine as an isotopic tracer, Biolo et al. observed that hyperinsulinemia within the leg increased blood flow and the rate of muscle protein synthesis without a substantial change in the rate of muscle protein breakdown or transmem-

brane transport of phenylalanine. Biolo et al. therefore concluded that insulin's anabolic action is mediated by direct stimulation of muscle protein synthesis rather than by an effect on transmembrane transport.

Critical illness is associated with a resistance to the action of insulin (6, 20). Yet despite insulin's retarded function during severe illness, several studies have nonetheless shown that insulin at pharmacological doses remains anabolic to muscle protein (18, 19). However, interpretation of insulin's mechanism of action in these prior studies has been confounded by the numerous systemic perturbations associated with hyperinsulinemia and/or the corrective maneuvers employed to maintain euglycemia, eukalemia, and other conditions. An accurate assessment of insulin's metabolic effect may be crucial in determining the process by which critical illness elicits insulin resistance. The purpose of this study was to quantify the effects of physiological hyperinsulinemia on skeletal muscle protein in critically ill, severely injured patients in a manner analogous to the previous study by Biolo et al. (3) in normal healthy volunteers and thereby to assess the mechanism by which insulin promotes anabolism in muscle and determine what metabolic alterations underlie any illness-induced resistance to insulin.

MATERIALS AND METHODS

Study subjects. Characteristics of the 14 study subjects are presented in Table 1. All patients had burns encompassing >40% of their body surface area and were admitted to the burn intensive care unit within 2 days of their injury. Subjects ranged in age from 23 to 52 yr and in weight from 54 to 130 kg. All subjects had good hemodynamics and adequate urine output at the time of study. Seven study subjects required mechanical ventilation, but no subject was hypoxic or acidotic during the study. Patients were excluded from participation if they were diabetic before their burn injury or had renal and liver dysfunction (serum creatinine >1.5 mg/dl, bilirubin >3.0 mg/dl). All patients were treated in a similar manner clinically with early excision of their burn wound and prompt skin grafting. Nutritional support was exclusively enteral (Vivonex TEN, Norvartis, Summit, NJ), initiated within 48 h after burn injury and delivered continuously during study via a nasal-jejunal tube. All subjects were receiving enteral nutrition at the time of study, with the desired caloric goal of 1.2 times their resting energy expenditure (REE). This volume of feed supplied an average 2,795 kcal/day, of which 84% of these calories were carbohydrates. This feeding formula delivers protein as free amino acids at a calorie-to-nitrogen ratio of 149:1, with patients receiving an average 106 g of protein per day.

Study protocol. This study was approved by the Institutional Review Board of The University of Texas Medical Branch, Galveston, TX. Informed written consent was obtained from a family

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Table 1. Patient demographics

Age, yr	37±2
Weight, kg	85±5
Leg volume, 100 ml	134±15
TBSA burn, %	54±4
REE/bpEE, %	1.42±0.04

Values are means ± SE of 12 men and 2 women. TBSA, total body surface area; REE and bpEE, resting and basal-predicted energy expenditures, respectively.

member before study. Metabolic measurements were performed between the 4th and 9th days after their burn injury. The study protocol for these measurements is diagrammed in Fig. 1 and was performed in each patient's burn intensive care unit room. Local anesthesia (1% lidocaine) facilitated placement of vascular access catheters into an adjacent femoral artery and vein. After baseline blood sampling, primed continuous infusion of d_5 phenylalanine ($2 \mu\text{mol/kg prime}$; $0.07 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) was given via central venous access and continued for the 8 h of study. Leg blood flow was determined by infusion of indocyanine green dye ($1 \text{ mg/min} \times 20 \text{ min}$) into the femoral artery with subsequent spectrophotometric analysis of blood drawn simultaneously from the femoral vein and from the peripheral or central vein access (16). Leg blood flow measurements were standardized for leg volume by integrating several circumference measurements with lengths of the calf, thigh, and foot. With use of local anesthesia, muscle biopsies were obtained from the vastus lateralis muscle with a Bergstrom needle. Muscle biopsies for basal period measurements were performed at the 2nd and 5th h of study. After 4.3, 4.45, and 5 h of isotopic infusion, blood was drawn simultaneously from the femoral artery and vein, thus completing the baseline metabolic measurements (basal period).

Immediately after basal-period measurements, insulin (Humulin, Eli Lilly, Indianapolis, IN) was infused into the femoral artery at $0.45 \text{ mIU}\cdot\text{min}^{-1}\cdot 100 \text{ ml leg volume}^{-1}$ for 3 h. Ten milliliters of each subject's blood were added to each infusate of insulin and rotated every 30 min to minimize binding of insulin to the plastic surface of the tubing and thus ensure the continuous delivery of insulin into the femoral artery. For requantification of leg blood flow, indocyanine green dye was infused for 30 min into the femoral artery, starting at 2.5 h of insulin administration. At 3 h of insulin infusion, blood was sampled from the femoral vein, and a muscle biopsy was obtained. The stopcock was then turned, thereby discontinuing the intra-arterial infusion of insulin, and a blood sample was immediately obtained from the femoral artery to complete insulin-period measurements.

Sample analysis. Blood glucose concentrations were quantified on a glucose/lactate 2300 Stat Analyzer (Yellow Springs Instruments, Yellow Springs, OH). Serum insulin concentrations were determined by commercial radioimmunoassay (Diagnostic Products, Los Angeles, CA).

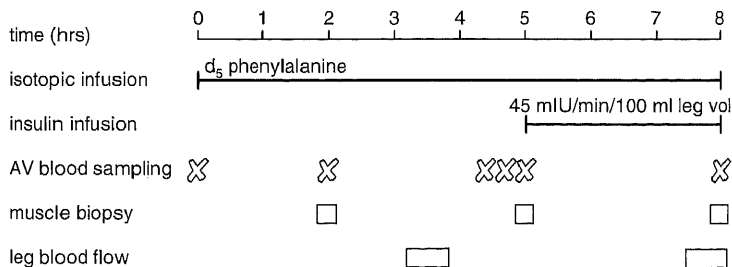


Fig. 1. Study protocol flow diagram. AV blood sampling, samples taken from femoral artery and vein.

Isotopic enrichment of phenylalanine and the blood concentration of unlabeled phenylalanine were determined by gas chromatography-mass spectrometry (GC-MS) by an internal standard method (23). Blood was collected in ice-cold tubes containing 2 ml of 15% sulfosalicylic acid and 200 μl of internal standard ($[^{13}\text{C}]$ phenylalanine). Samples were vortexed and centrifuged. The supernatant was then frozen at -80°C until processing. After *tert*-butyldimethylsilyl derivatization, plasma samples were then analyzed with GC-MS (model no. 5989; Hewlett-Packard, Palo Alto, CA) with electron impact ionization.

Muscle biopsies were analyzed for both intracellular and protein-bound amino acid concentration and isotopic enrichment with an internal standard method of analysis by GC-MS (9). To $\sim 20 \text{ mg}$ of muscle were added 800 μl of 14% perchloric acid and 2 μl of internal standard. Samples were homogenized and centrifuged, and the supernatant was collected. This procedure was repeated twice more and the pooled supernatant processed identically to the blood sample process just described by using *tert*-butyldimethylsilyl derivatization with analysis by GC-MS. This method determined the free intracellular concentration and isotopic enrichment for phenylalanine. For determination of protein-bound concentration and enrichment, the remaining muscle pellet was washed repeatedly with saline and absolute ethanol, dried, and then hydrolyzed with 6 N HCl. The protein hydrolysate was then passed over a cation exchange column (Dowex AG; Bio-Rad Laboratories, Richmond, CA), dried, esterified, heated, and subsequently analyzed by GC-MS with chemical impact ionization, as previously described (4).

Calculations. The net balance of phenylalanine across the leg was calculated as follows and was expressed per 100 ml leg volume

$$\text{AVNB} = ([\text{Phe}]_{\text{art}} - [\text{Phe}]_{\text{v}})\text{LBF}$$

where AVNB is net balance of phenylalanine across the leg ($\text{nmol}\cdot\text{min}^{-1}\cdot 100 \text{ ml leg volume}^{-1}$); $[\text{Phe}]_{\text{art}} - [\text{Phe}]_{\text{v}}$ is concentration of phenylalanine from artery and vein, respectively (nmol/ml); and LBF is leg blood flow ($\text{ml}\cdot\text{min}^{-1}\cdot 100 \text{ ml leg volume}^{-1}$). Thus a negative net balance reflects the net release of phenylalanine from the limb.

The kinetics of phenylalanine within skeletal muscle were calculated using a three-pool model, as previously described in detail (2) and illustrated in Fig. 2. This model allows quantification of amino acid transport into and out of muscle, as well as the rates of muscle protein synthesis and breakdown. Values are calculated as follows, and all are expressed as $\text{nmol}\cdot\text{min}^{-1}\cdot 100 \text{ ml leg volume}^{-1}$

$$F_{\text{in}} = [\text{Phe}]_{\text{art}} \times \text{LBF}$$

$$F_{\text{out}} = [\text{Phe}]_{\text{v}} \times \text{LBF}$$

$$F_{\text{ms}} = ([\text{IE}_{\text{m}} - \text{IE}_{\text{v}}]/(\text{IE}_{\text{art}} - \text{IE}_{\text{m}}))[\text{Phe}]_{\text{v}} + [\text{Phe}]_{\text{art}}\text{LBF}$$

$$F_{\text{vm}} = ([\text{IE}_{\text{m}} - \text{IE}_{\text{v}}]/(\text{IE}_{\text{art}} - \text{IE}_{\text{m}}))[\text{Phe}]_{\text{v}} + [\text{Phe}]_{\text{v}}\text{LBF}$$

$$F_{\text{va}} = F_{\text{in}} - F_{\text{ms}}$$

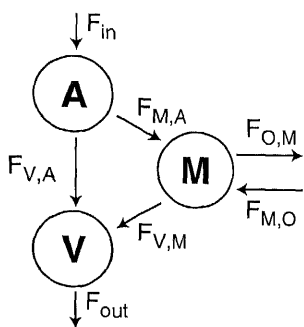


Fig. 2. Illustration of 3-compartment models for quantifying muscle protein kinetics within the leg. Free amino acid pools in femoral artery (A), femoral vein (V), and muscle (M) are connected by arrows indicating unidirectional amino acid flows between compartments. Amino acids enter leg via femoral artery (F_{in}) and leave leg via femoral vein (F_{out}). $F_{V,A}$, direct amino acid flow from artery to vein without entering intracellular fluid; $F_{M,A}$ and $F_{V,M}$, inward and outward amino acid transport from artery to muscle and from muscle to vein, respectively; $F_{M,O}$, intracellular amino acid appearance from endogenous sources (i.e., proteolysis); $F_{O,M}$, rate of disappearance of intracellular amino acids (i.e., protein synthesis).

$$F_{in} = F_{M,A}(IE_{M,A}/IE_{M,A} - 1)$$

$$F_{out} = F_{M,O} + AVNB$$

$$R_{in} = F_{M,A} + F_{M,O}$$

where F_{in} is flow of amino acid into leg; F_{out} is flow of amino acid out of leg; $F_{M,A}$ is flow of amino acid from artery into muscle; $F_{V,M}$ is flow of amino acid from muscle to vein; $F_{M,O}$ is flow of amino acid from artery to vein; $F_{M,A}$ is release of amino acid from bound protein to free concentration in muscle; $F_{M,O}$ is disappearance of amino acid from the intracellular pool; R_{in} is total rate of intracellular appearance of phenylalanine; and $IE_{M,A}$, $IE_{V,A}$, and $IE_{M,O}$ are isotopic enrichments of phenylalanine in arterial plasma, venous plasma, and muscle sample, respectively atom percent excess. Because phenylalanine is an essential amino acid in which production from the leg can come only from breakdown of muscle protein, $F_{M,O}$ for phenylalanine is a direct reflection of the rate of muscle protein breakdown. Likewise, $F_{M,A}$ for phenylalanine reflects the rate of muscle protein synthesis.

To index the efficiency of transmembrane transport with account taken for changes in leg blood flow and alterations in amino acid availability, the rate of amino acid inward transport ($F_{M,A}$) was normalized by the rate of amino acid delivery (F_{in}) and expressed as

$$F_{M,A}/F_{in}(\%) = \text{efficiency to transmembrane transport}$$

To index the efficiency of muscle protein synthesis with account taken for changes in leg blood flow and alterations in intracellular amino acid availability, the rate of muscle protein synthesis ($F_{O,M}$) was normalized by the rate of intracellular appearance for phenylalanine (R_{in}) and expressed as

$$F_{O,M}/R_{in}(\%) = \text{efficiency of muscle protein synthesis}$$

The three-pool model that we discussed quantifies muscle protein synthesis. However, as an independent measure of muscle protein synthesis, the fractional synthetic rate (FSR) of muscle protein was determined by quantifying the rate of incorporation of d_5 phenylalanine into muscle over time and calculated as follows (6)

$$FSR = [(IE_{p2} - IE_{p1})/(IE_m \times t)] \times 60 \times 100$$

Table 2. Response to insulin

	Basal Period	Insulin Period
Insulin concentration in femoral vein, $\mu\text{U/ml}$	50.5 ± 5.9	$128.3 \pm 10.4^{*†}$
Glucose concentration in femoral artery, mmol/l	11.9 ± 1.5	11.4 ± 1.4
Concentration of phenylalanine, mmol/l		
Femoral artery	45.2 ± 4.9	42.2 ± 3.8
Femoral vein	51.4 ± 6.0	45.7 ± 4.5
Muscle	130 ± 15	121 ± 10
Leg blood flow, $\text{ml} \cdot \text{min}^{-1} \cdot 100 \text{ ml leg volume}^{-1}$	4.73 ± 0.57	$6.40 \pm 1.39^{*}$

Values are means \pm SE. * $P < 0.05$; $†P < 0.01$ vs. basal.

where FSR is fractional synthetic rate (%/h), IE_{p2} and IE_{p1} are isotopic enrichments of protein-bound d_5 phenylalanine in muscle at times 1 and 2 (APE), IE_m is average isotopic enrichment in muscle (APE), t is time (min), and 60 and 100 are factors required to express FSR (%/h).

Statistics. Student's paired t -test was used for comparison between basal-period and insulin-period measurements. Results are presented as means \pm SE, and $P < 0.05$ was accepted as significant.

RESULTS

Study subjects were hypermetabolic as evidenced by the 42% elevation in REE (Tables 1 and 2). These burn patients were also protein catabolic, as illustrated by the negative net balance of phenylalanine from the leg, and hyperdynamic, as reflected by the increased leg blood flow above normal values (Tables 2 and 3). The femoral artery infusion of insulin resulted in a significant increase in the insulin concentration within the femoral vein (Table 2). Systemic perturbations from the insulin infusion were negligible, as evidenced by only a 4% decrease in the arterial concentration of glucose and a 7% decrease in the arterial concentration of phenylalanine (Table 2). Likewise, the concentration of phenylalanine within muscle was not significantly affected by the insulin infusion. There was a significant increase in leg blood flow associated with the insulin infusion (Table 2).

In regard to muscle protein kinetics, as calculated with the three-pool model, repeated measures taken during the final 30 min of both the basal and insulin study periods demonstrated a consistent isotopic enrichment for phenylalanine, thereby allowing use of steady-state equations. Insulin administration was associated with a significant increase in the inflow of phenylalanine into the leg (Table 3). Transport variables into and out of muscle, as well as net balance of phenylalanine

Table 3. Muscle protein kinetics by 3-pool model calculations

	Basal Period	Insulin Period
Net balance	-29 ± 6	-23 ± 4
Inflow (F_{in})	214 ± 46	$270 \pm 51^{*}$
Outflow (F_{out})	243 ± 40	$293 \pm 46^{*}$
Inward transport ($F_{M,A}$)	69 ± 11	77 ± 13
Release from muscle ($F_{M,O}$)	98 ± 21	99 ± 24
Shunt ($F_{V,A}$)	145 ± 27	$194 \pm 38^{*}$
Muscle breakdown ($F_{M,O}$)	53 ± 11	60 ± 12
Muscle synthesis ($F_{O,M}$)	24 ± 4	$38 \pm 5^{*}$
Intracellular appearance (R_{in})	122 ± 13	136 ± 13

Values are means \pm SE expressed as nmol of phenylalanine $\cdot \text{min}^{-1} \cdot 100 \text{ ml leg volume}^{-1}$. * $P < 0.05$ vs. basal.

across the leg, were not significantly affected by the insulin infusion. The shunt of phenylalanine from the artery directly to the vein was significantly increased by the insulin infusion. Although the rate of muscle protein breakdown as indexed by F_{mo} was not significantly affected by the insulin infusion, the rate of muscle protein synthesis as indexed by F_{om} increased significantly with the insulin infusion (Table 3). The rate of appearance of phenylalanine within muscle increased by 11%; however, this increase was not statistically significant. In response to the infusion of insulin, there was no significant change in the efficiency of transmembrane transport (F_{ma}/F_{in}) (Table 4). In contrast, the efficiency of muscle protein synthesis (F_{om}/R_{am}) significantly increased with the insulin infusion. Furthermore, the FSR of muscle protein increased by a significant 17% during insulin administration (Fig. 3).

DISCUSSION

Our goal with the intra-arterial infusion of insulin was to increase insulin availability within the leg with minimal systemic effects and thereby determine the isolated effect of insulin on human skeletal muscle protein *in vivo*. Insulin concentration with femoral venous sampling increased more than twofold, to an average value >120 mIU/ml, yet there was only a 4% decrease in the arterial concentration of glucose and only a 7% decrease in the arterial concentration of phenylalanine. Thus our protocol was generally successful in its intent. Our principal observation was that hyperinsulinemia stimulated muscle protein synthesis. With use of the three-pool model calculation, insulin mediated a nearly 60% increase in the rate of muscle protein synthesis. There was also a pronounced increase in leg blood flow and the flow of phenylalanine into the leg. Because determination of muscle protein synthesis by use of the three-pool model involves leg blood flow (2), we cannot exclude the possibility that the increase in blood flow affected the validity of this calculated value for muscle protein synthesis. Regardless of its determination, the FSR of muscle protein, which is based on the direct measurement of tracer incorporation into muscle and is thus independent of leg blood flow, increased a significant 17% with the infusion of insulin, thereby supporting the results of the three-pool model. The increase in protein synthesis was largely accomplished by an increased utilization of intracellular amino acids, as reflected by the increased efficacy of muscle protein synthesis as opposed to an accelerated influx from plasma. The rate of muscle protein breakdown was not significantly affected by insulin. Thus insulin's anabolic effect is predominantly mediated by an augmentation of muscle protein synthesis.

The action of insulin to increase muscle protein synthesis with neither a significant effect on amino acid transport nor an influence on the rate of muscle protein breakdown was evidenced previously in severely injured patients by Ferrando et

Table 4. Measures of efficiency for transmembrane transport and muscle protein synthesis

	Basal Period	Insulin Period
F_{ma}/F_{in} , %	32 ± 3	28 ± 3
F_{om}/R_{am} , %	20 ± 2	28 ± 3*

Values are means ± SE. F_{ma}/F_{in} , efficiency of transmembrane transport; F_{om}/R_{am} , efficiency of muscle protein synthesis. * $P < 0.05$ vs. basal.

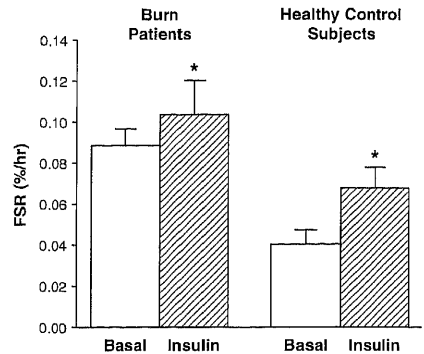


Fig. 3. Measures of fractional synthesis rate (FSR) for muscle protein. Values for healthy control subjects are derived from data in Ref. 3. * $P < 0.05$.

al. (8). Using a similar methodology, these investigators administered insulin intravenously at an average of 2.6 mIU·kg⁻¹·min⁻¹ to severe burn patients, resulting in a mean plasma insulin concentration of 242 mIU/ml. Like many previous investigations (1, 21), interpretation of the study by Ferrando et al. regarding the mechanism of action of insulin was confounded by a requirement that $>50\%$ of the study subjects received exogenous glucose to avert hypoglycemia. Another prominent complication from systemic insulin administration is a reduction in plasma amino acid concentrations, a factor that may have obscured the stimulatory effect of insulin on protein synthesis in several prior studies (1, 21). Whereas it is debatable whether or not exogenous insulin stimulates muscle protein synthesis in the basal state of normal subjects, there is little doubt that insulin stimulates muscle protein synthesis when amino acids are available in adequate quantity (24). Thus, in the context of both the present and prior studies, it is clear that in severely burned patients, who are considered insulin resistant in terms of insulin's effect on glucose, insulin is nonetheless effective in stimulating muscle protein synthesis.

One concern of this study is that the results are based on phenylalanine as the sole tracer for muscle protein response. Biolo et al. (3) showed that although phenylalanine transport

Table 5. Comparable measures from healthy subjects

	Basal Period	Insulin Period
Insulin concentration in femoral vein, μU/ml	10 ± 2	77 ± 9†
Leg blood flow, ml·min ⁻¹ ·100 ml leg volume ⁻¹	3.16 ± 0.39	4.12 ± 0.48*
Concentration of phenylalanine in muscle, mmol/l	124 ± 16	102 ± 15*
Shunt (F_{sa}), nmol·min ⁻¹ ·100 ml ⁻¹	68 ± 13	95 ± 16*
Muscle breakdown (F_{mo}), nmol·min ⁻¹ ·100 ml ⁻¹	56 ± 9	52 ± 6
Muscle synthesis (F_{om}), nmol·min ⁻¹ ·100 ml ⁻¹	40 ± 8	59 ± 8*
Efficiency of transport (F_{ma}/F_{in}), %	51 ± 2	48 ± 3
Efficiency of synthesis (F_{om}/R_{am}), %	31	44

Values are means ± SE and represent values from healthy subjects previously reported by Biolo et al. (3). In that study, insulin was infused at 0.15 mIU·min⁻¹·100 ml leg volume⁻¹, subject wt in kg was 73.5, and leg volume (100 ml) was 103 ± 7. Efficiency of synthesis values are calculated from the reported values, not reported directly; note the 42% increase from basal. * $P < 0.05$, † $P < 0.01$ vs. basal.

was not significantly altered by insulin, transmembrane transport of other amino acids (i.e., alanine, lysine, and leucine) was affected by insulin. Furthermore, *in vitro* work has shown an influence of insulin on several distinct transport systems involved in amino acid uptake, most notably N^m (14, 15). We have also recently shown that, in response to hyperglycemia, there is a wide discrepancy in metabolic flux within the leg of severe burn patients depending on the observed amino acid (13). Therefore, because of its status as an essential amino acid, phenylalanine may accurately index the kinetics of muscle protein; however, it is doubtful that phenylalanine universally reflects the metabolic response of other amino acids, especially in regard to the transport of other amino acids into and out of muscle. On the other hand, it is also possible that the failure of insulin to stimulate phenylalanine transport in this study may reflect a general deficiency of amino acid transport in severely ill patients. Another concern of this study was the discrepancy in magnitude of the protein synthetic response between the three-pool model (i.e., 60% increase) and the 17% increase evident by FSR. The measure of F_{om} for this study was determined at the end of a 3-h infusion of insulin, whereas the determination of FSR is an average rate of synthesis over the entire 3 h of insulin administration. One probable explanation for the smaller magnitude of insulin response by FSR is that there is a delay in the effect of insulin on muscle protein kinetics. Another possibility involves the unknown influence of leg blood flow on the calculated values of the three-pool model. In contrast, calculations for FSR are independent of leg blood flow. Therefore, the insulin-mediated increase in leg blood flow may have affected the magnitude of the calculated values for protein synthesis.

The demonstration by this study that local hyperinsulinemia increases muscle protein synthesis and leg blood flow in critically ill patients is very similar to that evidenced by Biolo et al. (3) in normal healthy subjects (Table 5). Thus insulin appears to affect muscle protein of critically ill patients and normal healthy subjects in the same general manner. However, although the response of insulin was similar, the quantity of insulin needed to produce this response was much greater (i.e., $\times 3$) in the severely burned patients. This finding could be interpreted as resistance to the action of insulin during critical illness. However, despite the greater volume of insulin infused into the leg of the burn patients, the magnitude of increase in the femoral venous concentration of insulin was comparable (i.e., 67 mU/ml for Biolo et al. and 77 mU/ml for the present study). This suggests that one component of illness-induced insulin resistance may result simply from an accelerated loss of insulin or a greater barrier in the binding of insulin to its membrane receptor. Accelerated whole body insulin clearance in burn patients has been previously well established (6), but this is the first demonstration that there could be increased peripheral clearance of insulin. In that regard, studies in patients with diabetes mellitus have found that a reduction in the transfer of insulin from capillary to tissue and an accelerated removal of insulin from tissue contribute to insulin resistance (22, 25). If the same is true in burn patients, then the interstitial edema and hyperdynamic state associated with adequately resuscitated critically ill patients may play a prominent but presently undetermined role in stress-induced insulin resistance.

The FSR of muscle protein increased significantly with the administration of insulin into the leg of the severely burned patients, but the increase was only 17%. In contrast, Biolo et al. (3) reported a 69% increase in FSR for healthy subjects (Fig. 3). Thus, despite a comparable increase in insulin availability within the leg, the magnitude of response from muscle protein was substantially blunted on a percentage basis in the critically ill patients. This could reflect a diminished effectiveness of insulin with severe illness. However, the basal FSR for the burn patients was already twice that of the basal rate for the healthy volunteers. Thus it is possible that the effectiveness of insulin as an anabolic agent diminishes as the rate of muscle protein synthesis approaches a maximum capacity. If true, then another aspect of insulin resistance may simply be an already heightened rate of muscle protein synthesis with critical illness. Counter to the measures of FSR, the three-pool model kinetic value from muscle protein synthesis (F_{om}) and the measure of efficiency for muscle protein synthesis (F_{om}/R_{am}) were less for the severely burned patients than those reported by Biolo et al. for normal volunteers (Table 5). However, the response of these measures to insulin was similar between healthy volunteers and burn patients (i.e., increase in F_{om}/R_{am} reported by Biolo was 43 vs. 39% for burn patients). This similarity in response to insulin despite the differences in absolute values further supports the contention that metabolic alterations related to critical illness (i.e., changes in extremity blood flow and amino acid availability), and not necessarily a diminished effectiveness to insulin, may underlie the observation of an illness-induced resistance to the action of insulin. Another possibility to explain the observed differences between the critically ill burn patients and the healthy subjects studied by Biolo et al. may be the ongoing nutritional support given the burn subjects, whereas the healthy subjects were studied while fasting. Prior work has shown that dietary supplementation of amino acids will augment amino acid transport into muscle with an associated increase in muscle protein synthesis (5). To what extent nutritional supplementation affects protein kinetic measurements by using the isolated limb of severely burned patients is unknown.

In conclusion, it is clear that, although the hypoglycemic action of insulin is diminished in burned patients, insulin nonetheless effectively stimulates muscle protein synthesis. Furthermore, this effect is direct and not dependent on concurrent changes in the availability of glucose or amino acids.

GRANTS

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