Insulin but not growth hormone stimulates protein anabolism in skin wound and muscle

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Insulin but not growth hormone stimulates protein anabolism in skin wound and muscle. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E712–E720, 1999.—We have measured protein kinetics in the scalded skin and normal muscle in anesthetized rabbits. On the 7th day after ear scald, L-[ring-13C6]phenylalanine was infused as a tracer, and the ear and hindlimb were used as arteriovenous units to reflect skin and muscle protein kinetics. Insulin was infused at 0.6 or 2.3–3.4 mU·kg⁻¹·min⁻¹ in the low-dose and high-dose insulin groups. In the growth hormone group, recombinant human growth hormone was administered at 2 mg·kg⁻¹·day⁻¹ after the ear was scalded. The results were compared with a control group in which the ear was scalded but otherwise was not treated. In the control group, net protein loss in the scalded skin and muscle was 23.1 ± 21.4 and 3.9 ± 1.5 µmol·100 g⁻¹·h⁻¹, respectively. Insulin infusion at either high or low dose reduced net protein loss to near zero by inhibiting proteolysis. In contrast, growth hormone treatment had no anabolic effect on either tissue. In conclusion, insulin but not growth hormone has an anabolic effect on scalded skin and normal muscle; low-dose insulin is as effective in achieving an anabolic effect on both tissues, with less hypoglycemic response than high-dose insulin.

Stable isotopes; mass spectrometry; arteriovenous balance; rabbit

Insulin and growth hormone (GH) have been extensively studied for their anabolic effects on protein metabolism in traumatized and septic patients. Previous studies from this institute support beneficial effects of both anabolic hormones. In burned patients, infusion of a pharmacological dose of insulin significantly decreased whole body protein breakdown and net protein catabolism (16) and eliminated net protein loss in the skeletal muscle (24). In septic patients, when somatostatin was infused to reduce basal insulin concentration, whole body protein synthesis decreased and net protein catabolism increased (29). Most recently, we have demonstrated that moderate doses of insulin are effective in eliciting the anabolic action on muscle protein metabolism in burned patients with minor hypoglycemic response (9). GH administration has been shown to stimulate whole body and muscle protein synthesis in postabsorptive patients (14) and donor site healing in burned patients (12, 15), but GH failed to stimulate muscle protein synthesis in patients already receiving insulin infusion (14). Others have also reported anabolic effects of insulin or GH in catabolic subjects (21, 22, 25, 26).

The effects of insulin and GH on wound protein metabolism have not been studied. Because a large open skin wound is the primary source of catabolic stimuli in burned patients and an early closure of the burn wound is the fundamental goal of acute burn treatment, knowledge of hormonal regulation of wound protein metabolism should provide important information in evaluating the benefits of anabolic hormones in burned patients. Because of the lack of methodology with which to determine protein metabolism in a skin wound, quantitative data of wound protein kinetics are not available. Recently, we have used the rabbit ear and stable isotope tracer techniques for the study of skin protein metabolism (28, 30). Because the ear exclusively represents skin protein metabolism and meets the requirements for application of the arteriovenous (a-v) balance method, the rabbit ear serves as a unique in vivo model for the study of skin protein metabolism. By creating a partial thickness scald injury on the ear, the ear model reflects protein metabolism in a skin wound.

The present study was designed to investigate metabolic effects of insulin and GH on protein kinetics in the scalded skin. The rabbit ear with a partial thickness scald injury was used as an a-v unit to reflect wound protein kinetics. Because the rabbits were studied under general anesthesia and surgical trauma (catheterization, tracheotomy), which we have previously shown to induce catabolic responses (28), negative results pertaining to wound protein metabolism could be attributed to either ineffectiveness of treatment or the anesthesia and surgery. To this end, we have simultaneously measured muscle protein metabolism by applying the a-v balance method to the hindlimb. Our previous study (28) has shown that the skeletal muscle of anesthetized rabbits responds to hormonal and nutritional perturbations, so that the muscle data served as a reference to evaluate the effectiveness of hormonal treatment.

METHOD

Animals. Male New Zealand white rabbits (Myrtle’s Rabbitry, Thompson Station, TN), weighing ∼4.5 kg, were used for this study. The rabbits were housed in individual cages and consumed Lab Rabbit Chow HF 5326 (Purina Mills, St. Louis, MO) for weight maintenance. This study was approved by the Animal Care and Use Committee of The University of Texas Medical Branch at Galveston.

Isotopes. L-[ring-13C6]phenylalanine (L-[ring-13C6]Phe; 99% enriched) and L-[ring-2H5]Phe (98% enriched) were purchased from Cambridge Isotope Laboratories (Woburn, MA). L-[ring-13C6]Phe was dissolved in 0.45% saline as a stock solution,
stored at 4°C, and then diluted with 0.9% saline into a working solution before each infusion. L-[ring-2H5]Phe was used to prepare an internal standard solution at a concentration of 30 μmol/ml for measurement of phenylalanine (Phe) concentrations in the blood.

Partial thickness thermal injury. After overnight fasting, the rabbits were anesthetized by intramuscular injection of ketamine (35 mg/kg) and xylazine (5 mg/kg). The left ear was shaved and disinfected with 70% isopropyl alcohol. The ear was submerged in 72°C water for 3 s. The exact scalding time was recorded using a stopwatch. This scalding procedure was selected from the pilot studies in which different water temperatures and scalding durations were tested. This scalding procedure consistently creates partial thickness thermal injury on the ear skin, as diagnosed by clinical and histological observations. After the scald injury, edema and redness developed on the entire ear, with blistering on the ventral side of the ear in each case. On the 7th day after burn, there were patches of open wound on the scalded ear; light microscopy demonstrated acute and chronic inflammation, increase in thickness and number of cell layers of epidermis, and small regions of epidermal ulceration. The wounds were completely healed during the third postinjury week. We selected the 7th day for the isotope infusion study because at that time the rabbits have recovered from the initial scald injury and there is partial thickness skin wound on the ear.

Immediately after the scald, a single dose of antibiotic (Bicillin; 50,000 U/kg; Wyeth Laboratories, Philadelphia, PA) was injected intramuscularly. On the animal's awakening, buprenorphine (0.03 mg/kg im) was injected twice daily as an analgesic for the first 3 days after the scald injury. The daily food consumption was recorded throughout the experimental period.

Experimental design. There were four groups: a control group (n = 10), a low-dose insulin (LDI) group (n = 5), a high-dose insulin (HDI) group (n = 5), and a GH group (n = 5). In the control group, the rabbits were not given any hormonal treatment before or during the isotope infusion period. In the LDI and HDI groups, insulin solution in albumin was infused at 0.6 or 2.3 mU·kg⁻¹·min⁻¹ and was started 1.5 h before the isotope infusion. A balanced amino acid (AA) solution (10% Travasol; Baxter Healthcare, Deerfield, IL) was infused at 0.6 ml·kg⁻¹·h⁻¹ with insulin to maintain blood AA concentrations constant. Blood glucose concentration was maintained at 5.5–6.0 mmol/l by variable infusion rates of glucose solution. The selection of the high-dose insulin was based on our previous study (28), in which the same dose of insulin was infused to investigate the response of protein metabolism in normal skin and muscle. The low-dose insulin was to determine whether there is dose-dependent effect of insulin on wound protein metabolism. In the GH group, recombinant human GH (Nutropin; Genentech, San Francisco, CA) was injected intramuscularly at 2 mg·kg⁻¹·day⁻¹ (1 mg = 31U) at 8:30 AM. This dose of GH was determined from the dose tests in which GH was injected intramuscularly at doses of 0 (saline as blank), 0.2, 1.0, 2.0, and 5.0 mg/kg, and serum GH and insulin-like growth factor I (IGF-I) concentrations were measured at 2, 4, 8, 12, and 24 h after GH injection. The results showed that the doses of 2 mg/kg were sufficient to maintain a high serum GH concentration for ≥12 h and to induce an increase in serum IGF-I level (Fig. 1).

The isotope infusion study was performed on the 7th day after the scald injury on the ear. After an overnight (16-h) fast with free access to water, the rabbits were anesthetized by intramuscular injection of ketamine and xylazine followed by continuous intravenous infusion of a mixture of these two anesthetics. Polyehtylene catheters (PE-90; Becton-Dickinson, Parsippany, NJ) were inserted in the right femoral artery and vein through an incision in the groin. The arterial line was used for blood collection and monitoring of heart rate and mean arterial blood pressure; the venous line was used for infusion. A tracheal tube was placed via tracheotomy. The central artery of the scalded ear and the left femoral artery were isolated for the placement of flow probes (model 1 RB for the ear artery and model 1.5 RB for the femoral artery; Transonic Systems, Ithaca, NY). The flow probes were connected to a small animal blood flowmeter (model T106, Transonic Systems) for measurement of blood flow rates (30). After completion of the surgical procedure, a blood sample was taken from the arterial line, and skin and muscle specimens were taken from the groin incision for background measurements. The infusion of L-[ring-13C6]Phe (0.15 μmol·kg⁻¹·min⁻¹; priming dose: 6 μmol/kg) was started after collection of the background samples in the control and GH groups. In the LDI and HDI groups, insulin and Travasol infusion was started first. Blood glucose concentration was measured every 10 min, and variable rates of glucose (D25) were infused to keep euglycemia. When the glucose infusion rate and blood glucose concentration were constant, which indicated a stable hyperinsulinemic euglycemic condition, the isotope infusion was started.

![Fig. 1. Changes of serum growth hormone (GH) and insulin-like growth factor I (IGF-I) levels after bolus injections of recombinant human growth hormone (rhGH). The dose of 2.0 mg/kg was sufficient to raise serum GH and IGF-I levels.](image-url)
During the 150–240 min of the tracer infusion, four arterial and ear-venous blood samples (0.6 ml each) and three arterial and femoral venous blood samples (0.4 ml each) were drawn alternatively at an interval of 10 min. The venous blood was drawn by directly puncturing the ear vein or femoral vein. The blood flow rate was recorded at each a-v blood sampling. At 240 min, a skin specimen was taken from the ventral side of the scalded ear, and a muscle specimen was taken from the biceps femoris of the experimental limb. Additional arterial blood was taken for measurement of plasma insulin concentration. Finally, both ears were cut off at the skin fold between the base and auricle to measure the ear weight.

The heart rate, mean arterial blood pressure, and rectal temperature were maintained stable by adjusting the infusion rates of anesthetics and physiological saline and by use of heating lamps. These vital signs were continuously monitored throughout the experiment and recorded every 30 min. The ear skin surface temperature was maintained at 37°C by means of a heating lamp.

Sample analysis. After the collection of blood samples, the internal standard solution, which contained 30 µmol/l of [L-\(^{2-\text{H}}\)Phe], was added to the blood (0.2 ml), and the samples were deproteinized by sulfosalicylic acid (30). The supernatants were processed to make the n-acetyl, n-propyl ester derivatives of the amino acids (27).

Plasma insulin concentration was determined by the Micro-particle Enzyme Immunoassay technique (1). Serum GH and IGF-I concentrations were measured by the radioimmunoassay and immunoradiometric assay, respectively, using assay kits (Nichols Institute Diagnostics, San J uan Capistrano, CA). Blood glucose concentration was measured on a glucose/lactate analyzer (model 2300, Yellow Spring Instruments, Yellow Springs, OH). Blood hemoglobin (Hb) concentration was measured on a Hemoglobin (HPLC) system (Waters 2690 HPLC system; Waters, Milford, MA) equipped with a Zorbax SB-C18 column.

Tissue specimens, either skin or muscle, of ~60 mg were processed for determination of free Phe enrichment. The tissues were homogenized in 5% perchloric acid three times at 4°C. The supernatant was processed to prepare the t-butylidimethylsilyl derivatives (10).

The isotopic enrichment of Phe in the blood was measured on a Hewlett-Packard 5985 gas chromatograph-mass spectrometer (GC-MS) (Hewlett-Packard, Palo Alto, CA) with chemical ionization. Ions were selectively monitored at mass-to-charge ratios of 250, 251, 255, 256 for Phe. Tissue free Phe enrichment was determined on a Hewlett-Packard 5980/5989B GC-MS; ions were selectively monitored at mass-to-charge ratios of 234, 235, 239, and 240 for Phe enrichment.

Isotope enrichment was expressed as a tracer-to-tracee ratio after correction for the contribution of the abundance of isotomers of lower weight to the apparent enrichment of isotomers with larger weight, and also a skew correction factor to calculate L-\(^{\text{ring-13C}_6}\)Phe enrichment (23).

Calculations. Protein kinetics and Phe transport in the scalded skin and normal muscle were calculated using a three-compartment model (5). The three pools are arterial pool (A), vein (V), and tissue (T) (Fig. 2). Further definitions necessary for the description of Phe kinetics are: 

- \(F_{\text{in}}\): the rate of Phe entering the a-v unit via arterial flow (i.e., inflow);
- \(F_{\text{T,A}}\): the rate of delivery from pool A to pool T (i.e., inward transport);
- \(F_{\text{V,A}}\): the rate of delivery directly from pool A to pool V (i.e., inward transport);
- \(F_{\text{V,T}}\): the rate of delivery from pool V to pool T (i.e., outward transport);
- \(F_{\text{T,O}}\): the rate of delivery from pool T to pool V (i.e., outward transport).

Data are expressed as means ± SD. Differences among the groups were evaluated using analysis of variance (ANOVA). A Student t-test with Bonferroni’s inequalities (12) was used for post hoc multiple comparison testing among the groups. \(P < 0.05\) was considered statistically significant.

RESULTS

During the first 48 h after scald, the rabbits took much less food than before the injury; thereafter, the food consumption gradually increased. On the 5th to 6th postinjury day, the rabbits had normal food consumption (150 g rabbit chow per day), except that two
rabbits in the GH group consumed only 50 and 110 g chow on the 6th day, respectively.

Table 1 lists the general characteristics of the rabbits. The body weight and exact scalld time were not significantly different among the groups. The weight ratio of scalded ear to contralateral ear was used as an indicator of wound edema; this ratio varied among individual rabbits but was not significantly different among groups. The rectal temperature was 1°C lower in the LDI and GH groups than in the control group (P < 0.05) during the sample collection period, whereas at the beginning of the experiment, the rectal temperature was not significantly different among groups (data not presented here). The skin wound surface temperature was kept at exactly 37°C in all the rabbits throughout the experiment. The LDI and HDI groups had significantly (P < 0.05 or P < 0.01) lower blood flow rates in the scalded skin than those in the control group. In the control group, leg a-v balance was measured in 5 of the 10 rabbits. Muscle blood flow rates were not significantly different among groups. The rabbits maintained a stable physiological condition, as the vital signs presented in Table 1 had relatively small percentages of coefficient of variation (CV) in individual rabbits: CV = 0.6 ± 0.6% for rectal temperature; CV = 4.4 ± 2.8% for heart rate; and CV = 4.5 ± 2.0% for mean arterial blood pressure (n = 25).

Because of variable wound edema, the weight of the scalded ear did not reflect skin mass. Thus the contralateral unscaled ear was used to estimate the skin mass in the ear: ear weight × 78%. Muscle mass in the leg was estimated by the body weight × 4.8%. The values of 78 and 4.8% were derived from the dissection of 10 ears and 10 legs in our previous study (28). When these values were used, the measured blood flow rates were converted to milliliters per 100 g per minute for both skin and muscle (see Table 1).

Substrate and hormone concentrations are presented in Table 2. There was a hyperglycemic response in the control and GH groups. During the insulin infusion periods, euglycemia was maintained by glucose infusion at 3.5 ± 2.0 and 8.1 ± 3.0 mg·kg⁻¹·min⁻¹ in the LDI and HDI groups, respectively. Plasma triglyceride concentration was significantly (P < 0.05) greater in the GH group than in the LDI and HDI groups. Serum free fatty acid concentrations did not reach significant difference among groups, although the GH group had the highest average value. Total plasma amino-N concentration was not significantly different among groups; plasma branched-chain amino acid (BCAA) and essential amino acid (EAA) concentrations were significantly (P < 0.05) lower in the HDI group than in the control group. Total nonessential amino acid (NEAA) concentration was significantly (P < 0.05) greater in the LDI group than in the control group. Plasma insulin concentration was 40 ± 36 pmol/l in the control group (n = 5). In the GH group, insulin concentration was 217 ± 177 pmol/l, which was not significantly different from either control or LDI group. Serum GH concentration was increased only in the GH group. Serum IGF-I concentration was significantly (P < 0.05) greater in the GH group than in the control and LDI groups.

Table 2. Substrate and hormone concentrations

<table>
<thead>
<tr>
<th>Study Group</th>
<th>n</th>
<th>Body Wt, kg</th>
<th>Scald Time, s</th>
<th>Core Temperature, °C</th>
<th>Heart Rate, beats/min</th>
<th>MAP, mmHg</th>
<th>Ratio of Scald to Normal Ear Wt</th>
<th>BF, ml·100 g⁻¹·min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>4.7 ± 0.3</td>
<td>3.2 ± 0.1</td>
<td>39.4 ± 0.9</td>
<td>199 ± 21</td>
<td>72 ± 8</td>
<td>1.6 ± 0.2</td>
<td>Scalded skin</td>
</tr>
<tr>
<td>LDI</td>
<td>6</td>
<td>4.7 ± 0.2</td>
<td>3.3 ± 0.1</td>
<td>38.4 ± 0.5</td>
<td>186 ± 21</td>
<td>66 ± 3</td>
<td>1.7 ± 0.2</td>
<td>Muscle</td>
</tr>
<tr>
<td>HDI</td>
<td>6</td>
<td>4.5 ± 0.7</td>
<td>3.3 ± 0.1</td>
<td>38.9 ± 0.3</td>
<td>214 ± 47</td>
<td>67 ± 5</td>
<td>1.4 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>GH</td>
<td>6</td>
<td>4.5 ± 0.3</td>
<td>3.1 ± 0.1</td>
<td>38.4 ± 0.4</td>
<td>193 ± 11</td>
<td>77 ± 15</td>
<td>1.6 ± 0.5</td>
<td></td>
</tr>
</tbody>
</table>

Data are means ± SD; n = 5 for each group. AA, amino acids; BCAA, branched-chain amino acid; EAA, essential amino acid; NEAA, nonessential amino acid; GH, growth hormone; IGF-I, insulin-like growth factor. *P < 0.05, †P < 0.01 vs. control; ‡P < 0.05 vs. LDI; §P < 0.01 vs. HDI.
During the 150–240 min of the isotope infusion period, arterial Phe enrichments reached plateaus (Fig. 3). Phe enrichment and concentration in the arterial and venous blood and Phe enrichment in the tissue free AA pool are presented in Table 3. The Phe concentrations in the ear venous blood have been corrected for water loss from the wound surface. Because of the impaired skin barrier, the scalded skin had faster water evaporation than normal skin. Thus the measured Phe concentration in the ear venous blood was higher than that in the arterial blood when the actual net balance was zero. To estimate the water loss from the wound surface, we measured the a-v difference in Hb concentration. Because Hb remains in the vascular compartment when water evaporates, its a-v difference reflects the water loss. We measured 20 paired arterial and ear venous blood samples for Hb concentration. Because Hb concentrations in the arterial and ear venous blood and Phe concentration from the 7-day scalded ear skin surface were 107.7 and 9.2 g/l, respectively, and the corresponding blood flow rate was 92 ml·100 g⁻¹·min⁻¹, these data mean that to account for the water loss from the wound surface, the measured venous Phe concentration should be multiplied by 98.3%. Furthermore, blood flow rate in the 7-day skin wound varied over a large range. Because the rate of water loss from the 7-day scalded ear skin surface should be constant under the same experimental conditions, the faster the blood flow, the smaller the a-v difference in Hb concentration, and vice versa. Therefore, we corrected the venous Phe concentration for the blood flow rate in each individual rabbit: correction factor = (1 − (92 × 1.7%/BF)), where 92 is the average blood flow rate (ml·100 g⁻¹·min⁻¹) at the time of collection of the a-v blood for Hb measurement; 1.7% is the average increase in Hb concentration from the artery to vein; and BF is the blood flow rate in the scalded skin.

Table 4 presents protein kinetic data in the scalded skin and normal muscle. The two doses of insulin infusion significantly (P < 0.05 or P < 0.01) improved net protein balance to a comparable extent in both scalded skin and normal muscle. The changes in the net balance were attributed to the decrease in protein breakdown, because the synthesis rates were not different. In the GH group, neither scalded skin nor normal muscle showed any anabolic responses in comparison with the control group.

Phe transport data are presented in Table 5. In the scalded skin, the rates of Phe inflow from the artery (F_in, see Fig. 2) were significantly (P < 0.05) smaller in the insulin groups than in the control group. The rates of inward transport (F_iA) were also smaller in the insulin groups (P > 0.05 LDI vs. control; P < 0.05 HDI vs. control, respectively).

Table 3. Phenylalanine enrichment and concentration in the 3 pools

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>EA</th>
<th>EV</th>
<th>ET</th>
<th>CA</th>
<th>CV</th>
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<tbody>
<tr>
<td>Ear balance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>0.1033 ± 0.0191</td>
<td>0.0971 ± 0.0194</td>
<td>0.0422 ± 0.0144</td>
<td>0.0738 ± 0.0098</td>
<td>0.0769 ± 0.0109</td>
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<tr>
<td>LDI</td>
<td>5</td>
<td>0.0962 ± 0.0117</td>
<td>0.0918 ± 0.0115</td>
<td>0.0417 ± 0.0046</td>
<td>0.0738 ± 0.0150</td>
<td>0.0745 ± 0.0147</td>
</tr>
<tr>
<td>HDI</td>
<td>5</td>
<td>0.0861 ± 0.0091</td>
<td>0.0838 ± 0.0091</td>
<td>0.0332 ± 0.0042</td>
<td>0.0696 ± 0.0059</td>
<td>0.0692 ± 0.0063</td>
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<tr>
<td>GH</td>
<td>5</td>
<td>0.1022 ± 0.0110</td>
<td>0.0956 ± 0.0095</td>
<td>0.0361 ± 0.0073</td>
<td>0.0666 ± 0.0129</td>
<td>0.0695 ± 0.0124</td>
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<tr>
<td>Leg balance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>0.0909 ± 0.0103</td>
<td>0.0703 ± 0.0072</td>
<td>0.0511 ± 0.0084</td>
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<td>LDI</td>
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<tr>
<td>GH</td>
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<td>0.0461 ± 0.0078</td>
<td>0.0661 ± 0.0150</td>
<td>0.0753 ± 0.0150</td>
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</table>

Data are means ± SD. Phe enrichment is expressed in a tracer-to-tracee ratio; Phe concentration is in µmol/ml. EA, EV, ET, arterial, venous, and tissue Phe enrichments, respectively; CA and CV, arterial and venous Phe concentrations, respectively.

Table 4. Protein kinetics

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Synthesis (µmol Phe·100 g skin⁻¹·h⁻¹)</th>
<th>Breakdown (µmol Phe·100 g skin⁻¹·h⁻¹)</th>
<th>Net Balance (µmol Phe·100 g skin⁻¹·h⁻¹)</th>
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</thead>
<tbody>
<tr>
<td>Ear balance</td>
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</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>26.6 ± 8.7</td>
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<tr>
<td>LDI</td>
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<td>21.8 ± 6.2</td>
<td>24.2 ± 5.1</td>
<td>−2.4 ± 1.8</td>
</tr>
<tr>
<td>HDI</td>
<td>5</td>
<td>20.5 ± 3.5</td>
<td>19.6 ± 3.4</td>
<td>1.0 ± 2.2</td>
</tr>
<tr>
<td>GH</td>
<td>5</td>
<td>25.6 ± 12.1</td>
<td>43.2 ± 23.2</td>
<td>−17.6 ± 11.9</td>
</tr>
<tr>
<td>Leg balance</td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>6.4 ± 2.7</td>
<td>10.3 ± 3.0</td>
<td>−3.9 ± 1.5</td>
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<tr>
<td>LDI</td>
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<td>−1.0 ± 0.4</td>
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<tr>
<td>HDI</td>
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<td>5.1 ± 1.6</td>
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<td>0.4 ± 0.4</td>
</tr>
<tr>
<td>GH</td>
<td>5</td>
<td>6.3 ± 1.4</td>
<td>10.0 ± 2.5</td>
<td>−3.1 ± 0.9</td>
</tr>
</tbody>
</table>

Data are means ± SD. *P < 0.05, **P < 0.01 vs. control; ***P < 0.05 vs. LDI; ****P < 0.05, *****P < 0.01 vs. HDI.
vs. control). Because of the smaller rates of protein breakdown and inward transport, the total Rₐ values in the wound free AA pool were significantly (P < 0.05 or P < 0.01) lower in the insulin groups than in the control group. Nevertheless, the ratio of synthesis to total Rₐ, an indicator of efficiency of protein synthesis, was greater in the insulin groups (P > 0.05 LDI vs. control; P < 0.01 HDI vs. control). The rates of outward transport (Fᵥ/𝑇) were significantly (P < 0.05) smaller in the insulin groups than in the control group. The ratio of inward transport to total Rₐ was not different among groups.

In the muscle, the rates of inward transport were not significantly different among groups. The decrease in total Rₐ in the insulin groups did not reach statistical significance when compared with either the control or the GH group. The insulin groups had significantly (P < 0.05 or P < 0.01) greater percent shunting and greater ratio of synthesis to total Rₐ (P < 0.05 LDI vs. control; P > 0.05 HDI vs. control). The rates of outward transport were significantly (P < 0.05) smaller in the insulin groups than in the control group.

Table 6 presents the rates of protein synthesis and breakdown in the scalded skin calculated from a two-pool model (3). There were no significant differences in protein synthesis rates. The insulin infusion significantly (P < 0.05 or P < 0.01) decreased the rates of protein breakdown in comparison with the control group. In the HDI group, the breakdown rate was significantly (P < 0.05) lower than in the LDI group.

**DISCUSSION**

In this study, the protein synthesis rate in the scalded skin varied over a range of 20–26 µmol·100 g⁻¹·h⁻¹, which is approximately twice the values we reported in the normal ear skin (8–15 µmol·100 g⁻¹·h⁻¹) (28). This indicates that the rate of protein turnover in the scalded skin was accelerated. In the control group, the increment of protein degradation exceeded that of synthesis, resulting in a substantial net protein loss in the scalded skin. The net protein loss was reduced to near zero in the insulin groups by mechanisms that inhibited proteolysis and maintained protein synthesis. The data of muscle protein metabolism showed responses to insulin infusion similar to those in the scalded skin. In contrast, GH administration did not change protein kinetics in either scalded skin or normal muscle. In the GH group, two rabbits had less food intake (50 and 110 g per day) than other rabbits (150 g per day). This cannot explain the catabolic results in this group, because these two rabbits did not have more negative protein balance than the other three in either scalded skin or normal muscle. In fact, the catabolic responses in the skin wound and muscle were consistent findings in all of the five rabbits in this group.

The results that insulin inhibited proteolysis in the scalded skin and normal muscle are consistent with our previous report (28), in which insulin infusion decreased protein breakdown rates in normal rabbit skin and muscle. Furthermore, in both studies, the decrease in breakdown resulted in improvement in net balance in skeletal muscle, because synthesis rate was not changed by insulin infusion in this study (see Table 4) and in our previous study (28). These findings support the notion that insulin infusion has an anabolic effect on muscle protein metabolism in the anesthetized rabbits with or without ear injury. Although protein degradation was also inhibited by insulin infusion in both normal and scalded skin, the response of protein synthesis was different. In normal skin, the rate of protein synthesis changed in parallel with the change in the breakdown rate in response to hyperinsulinemia. Thus the skin protein net balance remained zero. In contrast, in the scalded skin, insulin infusion also inhibited breakdown but did not significantly decrease the protein synthesis rate. Thus the net protein balance in the scalded skin was improved.

We have previously reported that, in healthy volunteers, local hyperinsulinemia stimulated the rate of
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Nevertheless, insulin may stimulate muscle protein synthesis, which was accompanied by increased leg blood flow rate (6). However, in the current study, as well as in our previous study of the rabbit (28), insulin infusion did not stimulate either muscle protein synthesis or muscle blood flow rate.

This discrepancy is probably due to the differences in experimental conditions. Baron et al. (2) have concluded that insulin-mediated vasodilation is an important physiological determinant of insulin action on muscle glucose uptake. When they infused NG-monomethyl-L-arginine into the femoral artery to blunt the insulin-mediated vasodilation, the leg glucose uptake decreased. The same mechanism may be applicable to leg Phe uptake, as in our previous studies in resting human volunteers we have found significant correlations between leg blood low and inward AA transport and between blood flow and protein synthesis (6, 7). Even if blood flow is not an independent regulator of Insulin-stimulated glucose uptake, as was demonstrated by Nuutila et al. (20), the lack of vascular response to insulin infusion in the present study (see Table 1) and in our previous study (28) indicates a modified insulin action in the anesthetized rabbits. In general, the stimulatory effect of insulin on the capacity of muscle protein synthesis requires an abundant supply of AA to be reflected in an increased amount of protein produced (22). In this experiment, the plasma concentrations of total amino-N were similar in all groups and corresponded to normal postabsorptive levels. In the LDI group, although plasma total NEAA concentration was significantly (P < 0.05) greater than in the control group, total EAA concentration was not increased. In the HDI group, total BCAA and EAA concentrations were significantly (P < 0.01) lower than that in the control group (see Table 5). Consequently, the rate of inward transport (FV,T) was lower (P < 0.05 LDI vs. control; P < 0.01 HDI vs. control), an indicator of efficiency in protein synthesis. In the insulin groups, 50–65% of wound free Phe was used for protein synthesis, which was in contrast to the values of 36–38% in the control and GH groups. As a consequence of increased efficiency of protein synthesis, the rates of outward transport (FV,T) were significantly (P < 0.05) smaller in the insulin groups than in the control group. Although the response of skin blood flow to insulin is not well documented, in other tissues insulin stimulates blood flow. The decreased wound blood flow rate in the insulin groups was not likely a direct effect of insulin. Rather, the decreased wound blood flow was mediated by other factors. Insulin might downregulate the wound inflammatory reaction, and thus both wound blood flow and wound proteolysis were decreased.

In the GH group, serum GH and IGF-I concentrations were significantly higher than in other groups. Plasma insulin, triglycerides, free fatty acids, and blood glucose levels also tended to be higher (see Table 2). This hormone and substrate profile has been consistently found in GH-treated patients (14, 15, 17, 19, 26) and animals (18), indicating that the rabbits were sensitive to GH administration. However, neither scalded skin nor normal muscle had an anabolic response to the GH treatment. This could be due to the insufficiency of insulin in reversing hyperglycemia and protein catabolism in the GH group. GH is known to induce insulin resistance to peripheral glucose uptake (4, 11, 26), and an increased insulin concentration is a common finding in all GH-treated subjects (4, 11, 14, 15, 18, 26). In those studies, exogenous insulin was often also given to control the hyperglycemia resulting from the GH. Even if insulin was not infused, hyperglycemia caused increased insulin concentration, as it did in the current study. Thus the increased insulin concentration could be responsible for the observed anabolic effects of GH on protein metabolism, as insulin effectively stimulated protein anabolism in burned and septic patients (16). In that light, in the current study it is not clear why the increase in insulin caused by GH did not induce an anabolic response, because the same approximation concentration of insulin resulting from insulin infusion was effective. It is possible that the increase in insulin during GH was insufficient to overcome the insulin resistance caused by the GH. The extent of the insulin resistance in the GH group is evidenced by the high glucose concentration, even in the presence of elevated insulin concentration.

In this study, we used tissue free Phe enrichment as an approximation of labeling of the aminoacyl-tRNA. In the scalded skin, the free AA pool could contain a large portion of interstitial fluid. Because the Phe tracer enters the intracellular pool from the vascular compartment, and the interstitial fluid is located between blood vessels and the intracellular pool, Phe enrichment in the interstitial pool should be lower than blood enrichment but higher than intracellular enrichment. Thus the inclusion of interstitial fluid in the skin sample could only overestimate the true precursor enrichment.

In the control group, the blood flow rate in the scalded skin was 115 ± 34 ml·100 g⁻¹·min⁻¹ (see Table 1), which was 15-fold that in normal skin (7.7 ml·100 g⁻¹·min⁻¹; see Ref. 28). The wound blood flow rates in the insulin groups were decreased by 41–44% in comparison with the control group. Thus the rate of Phe inflow from the artery to the wound free AA pool (Fv,in in Fig. 2) was significantly (P < 0.05) less than that in the control group (see Table 5). Consequently, the rate of inward transport (Fv,T) was lower (P > 0.05 LDI vs. control; P < 0.05 HDI vs. control). When coupled with the decreased rate of Phe breakdown, total Ra in the wound free AA pool was significantly (P < 0.05 or P < 0.01) smaller in the insulin groups than in the control group. However, the protein synthesis rates were not significantly decreased despite the decreased AA availability. This could be explained by the increased ratio of synthesis to total Ra (P > 0.05 LDI vs. control; P < 0.01 HDI vs. control), an indicator of efficiency in protein synthesis. In the insulin groups, 50–65% of wound free Phe was used for protein synthesis, which was in contrast to the values of 36–38% in the control and GH groups. As a consequence of increased efficiency of protein synthesis, the rates of outward transport (FV,T) were significantly (P < 0.05) smaller in the insulin groups than in the control group. Although the response of skin blood flow to insulin is not well documented, in other tissues insulin stimulates blood flow. The decreased wound blood flow rate in the insulin groups was not likely a direct effect of insulin. Rather, the decreased wound blood flow was mediated by other factors. Insulin might downregulate the wound inflammatory reaction, and thus both wound blood flow and wound proteolysis were decreased.

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Thus the values of protein synthesis, breakdown, and intracellular kinetics in the scalded skin presented in this article should be considered as minimal estimates of the true values. In any case, conclusions regarding net protein balance in the scalded skin are independent of this issue. Even if we used a two-pool model (3) based entirely on blood analysis, the responses of protein kinetics to insulin and GH (see Table 6) are similar to those calculated from the three-compartment model. When the two-pool model was used, the rate of protein breakdown in the scalded skin was further inhibited in the HDI group in comparison with the LDI group, whereas the three-pool model did not show significantly different breakdown rates between these two groups. This is because in the HDI group there was more intracellular cycling of Phe, which efficiently utilized AA released from proteolysis for synthesis (see Table 5).

In summary, insulin has an anabolic effect on protein metabolism in the scalded skin and normal muscle of anesthetized rabbits. Insulin inhibits accelerated wound proteolysis and wound blood flow, suggesting a possible downregulation of the wound inflammatory response. Low-dose insulin infusion is effective in eliciting the anabolic effect with a minor hypoglycemic response. In contrast, GH administration does not have anabolic effect with a minor hypoglycemic response. In anesthetized rabbits, insulin inhibits accelerated wound proteolysis and wound blood flow, suggesting a possible downregulation of the wound inflammatory response.

We conclude that insulin, but not GH, has a direct anabolic effect on protein metabolism in the scalded skin and normal muscle.

We are grateful to Dr. Hal K. Hawkins for histological diagnosis on the scalded skin and Mark D. Thompson for the hormone assay. We also thank the staff in the Clinical Laboratory of Shriners Hospital for measurement of blood hemoglobin concentration, and the staff in the Animal Resource Center of the University of Texas Medical Branch for professional care of the animals. We acknowledge the excellent technical assistance of Guy J ones, Yunxia Lin, and Zhanpin Wu.

This work was sponsored by Shriners Hospital for Children Grants 8630 and 8490.

This study was presented in abstract form at the Experimental Biology Meeting, San Francisco, CA, April 18–23, 1998.

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Received 25 August 1998; accepted in final form 30 November 1998.

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