Growth hormone decreases muscle glutamine production and stimulates protein synthesis in hypercatabolic patients

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Received 13 August 1999; accepted in final form 24 February 2000

Am J Physiol Endocrinol Metab

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PATTERNS SUFFERING FROM MAJOR INJURY have a rapid and progressive loss of skeletal muscle protein that can be reduced only partly by nutritional support (5, 14, 30). The administration of recombinant human growth hormone (rhGH) as adjunctive therapy has been shown to be effective in slowing the loss of muscle mass in patients (8, 16, 17, 28, 37, 38), despite the fact that it may have some secondary harmful effects (31, 37). Changes in protein mass derive from a balance between protein synthesis and degradation, which in turn can be influenced by the rate of transmembrane amino acid transport. Evidence indicates that stimulation of protein synthesis is a primary mechanism of rhGH action on muscle (12, 13). However, the hormone effects on protein degradation and amino acid transport have not been clarified.

Besides an anticatabolic effect, rhGH action on muscle also involves a suppression of glutamine efflux, which is much greater than that expected on the basis of the hormone’s effects on protein metabolism (4, 28). Such a decrease in glutamine efflux can result from changes in glutamine de novo synthesis and/or outward transmembrane transport. Glutamine is the most abundant free amino acid in the body. It is a precursor of many compounds (e.g., glucose, glutathione, and nucleic acids) and a major fuel for rapidly dividing cells (intestinal mucosa, immune system, and wound tissue). Glutamine is synthesized primarily in skeletal muscle and released into the bloodstream to serve as an interorgan vehicle for carbon and nitrogen. The key enzyme for glutamine synthesis is glutamine synthetase, whereas precursor substrates are glutamate, α-ketoglutarate, free ammonia, and amino-nitrogen derived from the catabolism of the branched-chain amino acids (9). In skeletal muscle, there is a large intracellular pool of preformed free glutamine that serves as a reservoir for any increased glutamine requirement in extramuscular tissues. A decline in muscle free glutamine has consistently been observed in trauma patients (5).

The aim of this study was to define the mechanisms of the rhGH-mediated changes in muscle glutamine and protein kinetics in severely traumatized patients during combined enteral and parenteral nutrition. Leg arteriovenous catheterization, muscle biopsy, and stable isotopic tracer of amino acids were used to determine the rates of muscle protein synthesis, proteolysis, glutamine de novo synthesis, nonprotein utilization of glutamate (mainly to form glutamine) and the branched-chain amino acids (catabolism), as well as transmembrane transport of glutamine and selected essential amino acids (2–4, 6). Competitive RT-PCR was used to determine muscle mRNA levels of key...
enzymes for glutamine synthesis (glutamine synthetase) and for myofibrillar (ubiquitin) and nonmyofibrillar (cathepsin B) protein degradation (7, 15, 27, 34, 35).

**METHODS**

**Patients**

Eight adult patients (6 males, 2 females; age 39 ± 5 yr, weight 74 ± 4 kg, height 172 ± 3 cm) with multiple injuries (APACHE II score 15 ± 1) were studied between days 7 and 12 after admission to the Intensive Care Unit of the University Hospital of Cattinara, Trieste, Italy. Leg volume (8,603 ± 520 ml) was estimated by use of an anthropometric approach (21). Informed consent was obtained from the patients’ close relatives. The protocol was approved by the competent hospital authority. All patients received continuous combined intravenous (80% of total energy as amino acids, glucose, and lipids) and enteral (20% of total energy: Nutrisond (Nutricia, Zoetermeer, Netherlands) or Sondalis (Bergstrom, Woburn, MA) was started, followed at hour 24 by L-[ring-2H3]phenylalanine (MassTrace) and L-[1-13C]leucine (MassTrace). Tracer infusions were maintained constant throughout the experiment. The following tracer infusion rates (IR) and priming doses (PD) were used: L-[ring-2H3]phenylalanine: IR = 0.05 μmol·kg⁻¹·min⁻¹, PD = 2 μmol/kg; L-[1-13C]leucine: IR = 0.08 μmol·kg⁻¹·min⁻¹, PD = 4.8 μmol/kg; L-[5,15N]glutamine: IR = 0.35 μmol·kg⁻¹·min⁻¹, PD = 63 μmol/kg. Isotope infusions were not started simultaneously, because the equilibration period of each tracer varied (2). L-[5,15N]glutamine required 5 h to reach steady state in muscle (2). L-[ring-2H3]phenylalanine and L-[1-13C]leucine were infused for 3 h.

To measure leg blood flow, at hour 23 a primed continuous infusion of indocyanine green dye (IR = 0.5 mg/min; PD = 5 mg) into the femoral artery was started and maintained for 30 min. During the last 15 min of indocyanine green infusion, four blood samples were taken every 5 min from the femoral and left antecubital vein for spectrophotometric determination of steady-state dye serum concentrations. The rate of leg plasma flow was calculated at steady state from the ratio between the dye infusion rate and the difference between serum dye concentrations in femoral and antecubital veins. Leg blood flow was calculated from the hematocrit. During the last 30 min of the intra-arterial rhGH or saline infusion, four blood samples were taken every 10 min from the femoral artery and vein to determine amino acid enrichments and concentrations. To allow sampling from the femoral artery, the rhGH or the saline infusion was stopped for <10 s and then quickly resumed. Blood samples were also taken from the femoral and left antecubital veins to determine local and systemic growth hormone concentrations, respectively. Insulin and insulin-like growth factor I (IGF-I) concentrations were measured in blood samples taken from the left antecubital vein. At hour 24, after the last blood sample was taken and before stopping the tracer and the rhGH or saline infusions, a muscle biopsy was taken to measure enrichments and concentrations of free amino acids and mRNA levels of cathepsin B, ubiquitin, and the glutamine synthetase enzyme. The biopsy was taken from the lateral portion of the vastus lateralis muscle ~20 cm above the knee with a Bergström biopsy needle (Stille, Stockholm, Sweden) (2–4, 6). Approximately 80–100 mg of muscle tissue were obtained with each biopsy. This procedure yields a sample of mixed skeletal muscle. Blood, visible fat, and connective tissue were quickly removed from the specimen, and the tissue was immediately frozen in liquid nitrogen and stored at ~80°C for later analysis. Thereafter, in the four patients previously infused with saline, an rhGH infusion was started into the femoral artery and continued for 24 h. In the other four patients previously treated with rhGH, a 24-h saline infusion was commenced. During the last 5 h of either rhGH or saline administration, tracer infusion was performed, leg blood flow was measured, and blood samples and muscle biopsies were taken, as described.

**Amino Acid Enrichments and Concentrations**

Blood samples taken from the femoral artery and vein to determine amino acid enrichments and concentrations were collected in preweighed tubes containing 1% saponin. Simultaneously, a known amount of a homoserine solution was obtained to measure background amino acid enrichments and indocyanine green concentration. Then a primed continuous infusion of L-[5,15N]glutamine (MassTrace) required 5 h to reach steady state in muscle (2). L-[ring-2H3]phenylalanine and L-[1-13C]leucine were infused for 3 h.
added to the tube as internal standard and thoroughly mixed. The blood was then precipitated with 15% sulfosalicylic acid (SSA). The tubes were weighed again, and the difference was recorded as blood volume after subtraction of the internal standard and SSA volumes. The supernatant was frozen for later analysis. To determine tracer enrichments, the t-butyldimethylsilyl derivatives were prepared as described (2).

Each tissue sample was weighed, and muscle protein was precipitated with 15% SSA. A known amount of a homoserine solution was added as internal standard and thoroughly mixed. The tissue was then homogenized and centrifuged, and the supernatant was collected. This procedure was repeated twice more. The pooled supernatant was frozen for later analysis. Muscle and whole blood SSA extracts were processed to determine free amino acid enrichments and concentrations by gas chromatography-mass spectrometry (Incos XL, Finnigan, Bremen, Germany) and HPLC (Beckman, Berkeley, CA), respectively. Mass spectrometry analysis was performed by electron impact ionization and selected ion monitoring for the t-butyldimethylsilyl derivatives of leucine (mass-to-charge ratios (m/z) 302 and 303), phenylalanine (m/z 234 and 239) and glutamine (m/z 431 and 432). Data were expressed as a tracer-to-tracee ratio. Concentrations (nmol/ml) of free amino acids in blood and total muscle water were calculated as referenced (2). Measured values of enrichment and concentrations relative to total tissue water were corrected (2, 18) to obtain intracellular values. These corrections required the knowledge of amino acid concentration and enrichment in the interstitial fluid, as well as knowledge of the proportion between intra- and extracellular water in muscle. We assumed that amino acid enrichment and concentrations in the interstitial fluid equaled blood values in the femoral vein and that the ratio between intra- and extracellular water in muscle was 0.16, as previously determined in humans by the chloride method (2).

**Calculation of Kinetic Parameters**

At the whole body level, amino acid rates of appearance (R_a) were calculated by dividing isotope infusion rates by arterial enrichments. Because amino acid intakes were identical in the control studies and during rhGH infusions, changes in the R_a of the essential amino acids phenylalanine and leucine can be considered as markers of changes in whole body proteolysis. Whole body glutamine clearance was calculated by dividing the glutamine R_a by the arterial glutamine concentration.

The net leg balance for amino acids was calculated from the Fick principle

\[ \text{net balance} = (C_A - C_V) \cdot BF \]  

where C_A and C_V are whole blood amino acid concentrations in femoral artery and vein, respectively; BF is leg blood flow. A positive value indicates net uptake, whereas a negative value indicates net release. Skeletal muscle is considered to account largely for amino acid metabolism in the whole leg (2). In the steady-state condition of muscle free amino acid concentrations, amino acid uptake or release across the leg reflects the balance between intracellular production and disposal for that particular amino acid. Thus net phenylalanine, tyrosine, and lysine release from leg muscle are markers of net protein catabolism, because these amino acids are not synthesized or oxidized in muscle tissue (1, 26, 36). Furthermore, phenylalanine is not hydroxylated to tyrosine in muscle (1, 26, 36), whereas the kidney and the splanchnic bed together account for all of the whole body hydroxylation of this amino acid (32). In contrast, skeletal muscle is the main site of catabolism of the branched-chain amino acids leucine, valine, and isoleucine, and of synthesis of alanine from pyruvate and of glutamine from glutamate. We assumed that amino acids are released from proteolysis in proportion to their relative content in muscle protein (2–4, 6, 24). Thus the net rates of release from protein catabolism of glutamate, glutamine, alanine, and the branched-chain amino acids can be calculated from the net rate of phenylalanine release, corrected for the molar ratios glutamate/phenylalanine (1.87), glutamine/phenylalanine (2.05), alanine/phenylalanine (2.35), leucine/phenylalanine (3.10), isoleucine/phenylalanine (2.55), and valine/phenylalanine (1.77) determined in mixed human muscle protein (2, 24). Then, the rates of net alanine and glutamine synthesis (i.e., the differences between the rates of synthesis and nonprotein utilization of the amino acids) of net nonprotein disposal of glutamate (mainly to form glutamine) and of branched-chain amino acid catabolism can be calculated by subtracting from the total release or uptake of these amino acids the component accounted for by protein catabolism.

\[
\begin{align*}
\text{net alanine synthesis} &= - (\text{net alanine balance}) \\
\text{net glutamine synthesis} &= - (\text{net glutamine balance}) \\
\text{net nonprotein glutamate disposal} &= \text{[net glutamate balance]} \\
\text{leucine catabolism} &= \text{[net leucine balance]} \\
\text{isoleucine catabolism} &= \text{[net isoleucine balance]} \\
\text{valine catabolism} &= \text{[net valine balance]} \\
\text{total branched-chain amino acid catabolism} &= \text{leucine catabolism + isoleucine catabolism + valine catabolism}
\end{align*}
\]

This calculated rate of branched-chain amino acid catabolism (i.e., nonprotein branched-chain amino acid disposal) is not equivalent to the actual rate of branched-chain amino acid oxidation; in fact, branched-chain amino acids are deaminated to the corresponding ketoacids before undergoing irreversible decarboxylation and oxidation. Some of these ketoacid molecules could escape intracellular metabolism and be released into the bloodstream. In our study, we did not measure the arteriovenous balance of branched-chain ketoacids across the leg to directly determine branched-chain amino acid oxidation. Nonetheless, the difference between the rates of branched-chain amino acid catabolism and oxidation is likely to be very small for two reasons. First, the arteriovenous difference across the leg of the leucine ketoacid α-ketoisocaproate is usually very small (33). Second, during leucine tracer infusion, there is a net uptake across the leg of the α-ketoisocaproate tracer derived from deamination of the leucine tracer (33).

Inward and outward amino acid transports are calculated as the rates of net amino acid movements from the femoral artery to muscle and from muscle to the femoral vein (2, 3, 6), respectively.
inward amino acid transport = \frac{[\{E_M - E_V\}/(E_A - E_M)]}{C_V + C_A} \times BF \quad (9)
\[
\text{outward amino acid transport} = \frac{[\{E_M - E_V\}/(E_A - E_M)]}{C_V + C_V} \times BF \quad (10)
\]
where \(E_A\), \(E_V\) and \(E_M\) were amino acid enrichments in the femoral artery, femoral vein, and muscle, respectively.

Muscle protein synthesis and proteolysis were calculated as rates of intracellular phenylalanine disposal and appearance (2, 3, 6), respectively.

**Determination of Specific mRNA Levels**

Muscle mRNA levels of cathepsin B (7), UbB polyubiquitin (7, 35), and glutamine synthetase (34) were determined by competitive RT-PCR (15). Ubiquitin is encoded in the human genome as a multigene family (35). Among the different ubiquitin genes, we assessed the UbB polyubiquitin gene, which codes for three direct repeats of the ubiquitin sequence, including the middle, was constructed by two separate PCR amplifications. The latter is calculated from the rate of intracellular phenylalanine appearance from proteolysis (Eq. 7) and the molar ratio glutamine/phenylalanine in mixed muscle proteins (2)

\[
\text{glutamine de novo synthesis} = \frac{[\{C_{\text{AGLN}} \times E_{\text{AGLN}} - C_{\text{VGLN}} \} \times E_{\text{VGLN}}/E_{\text{MGLN}}]}{[\{E_{\text{AGLN}} \times E_{\text{AGLN}} - C_{\text{VGLN}} \} \times E_{\text{VGLN}}/E_{\text{MGLN}}]} - \frac{[\{C_{\text{AGLN}} \times E_{\text{AGLN}} - C_{\text{VGLN}} \} \times E_{\text{VGLN}}/E_{\text{MGLN}}]}{[\{C_{\text{AGLN}} \times E_{\text{AGLN}} - C_{\text{VGLN}} \} \times E_{\text{VGLN}}/E_{\text{MGLN}}]} \times 2.05 \times BF \quad (13)
\]

where (GLN) indicates the values of glutamine concentrations and enrichments.

Quantitative PCR amplifications were performed by mixing scalar amounts of competitor DNA to the target cDNA followed by PCR amplification with the appropriate primer pairs. All amplifications were conducted in PCR buffer containing the two primers, the four dNTPs, 2.5 μU of Taq DNA polymerase (Boehringer), 1 μl of cDNA, and 1 μl of appropriately diluted competitor DNA, using a DNA Thermo Cycler (Perkin-Elmer Cetus). Samples were subjected to 38 cycles of amplification. After amplification, PCR products were resolved on an 8% non-denaturing polyacrylamide gel, visualized under ultraviolet light after ethidium bromide staining, and photographed. Quantification of the amplification products was obtained by densitometric scanning on photographs of the ethidium bromide-stained gels. According to the principles of competitive PCR, the ratio between the amount of the PCR products for the target cDNA and that of the competitor DNA is linearly correlated with the initial amount of cDNA in the reaction. To verify such a linear correlation, we plotted the densitometric ratios between the PCR products of scalar amounts of competitor DNA (0 pg, 0.01 pg, 0.05 pg, 1 pg, 2 pg) and fixed amounts of target glutamine synthetase cDNA with the initial amount of competitor DNA in the reaction. The correlation coefficient \(r^2\) was 0.99989, and the equation was \(y = 2.55 \times +0.04\).

Cathepsin B, UbB polyubiquitin, and glutamine synthetase mRNA levels were calculated from the ratios between cathepsin B and GAPDH cDNA, between UbB polyubiquitin and GAPDH cDNA, and between glutamine synthetase and GAPDH cDNA quantities in the same sample. The units of cathepsin B, UbB polyubiquitin, or glutamine synthetase mRNA content are expressed as %GAPDH mRNA.
levels. It is assumed that rhGH administration does not modify GAPDH mRNA levels in skeletal muscle of trauma patients (19, 25). The variation coefficient for GAPDH mRNA content for four different samples from the same muscle specimen was 3.2% of the mean when the samples were processed on the same day and 4.3% of the mean when the samples were processed on different days. The two muscle samples from a single subject, whether studied in basal conditions or during rhGH infusion, were always processed together.

**Data Presentation and Statistics**

All data are expressed as means ± SE. Because the values of leg blood flow, amino acid concentrations, and kinetics were not significantly different in the control studies performed before rhGH infusion (protocol 1) and 24 h after rhGH discontinuation (protocol 2), the results obtained in the two control studies were pooled together. Then, the values of leg blood flow, amino acid concentrations, and kinetics obtained during rhGH infusions (protocols 1 and 2) were compared with those in the control studies (protocols 1 and 2) by means of Student's t-test for paired samples. The effects of rhGH infusion (protocol 1) and rhGH discontinuation (protocol 2) on muscle levels of selected mRNAs were assessed separately in the two protocols by means of Student's t-test for paired samples. *P* < 0.05 was considered statistically significant.

**RESULTS**

Before rhGH infusion, plasma growth hormone concentration was 3 ± 1 ng/ml (protocol 1) and increased to 91 ± 9 ng/ml in the femoral venous and to 39 ± 3 ng/ml in the antecubital vein during rhGH infusion (protocols 1 and 2). After rhGH discontinuation, plasma growth hormone concentration decreased to 3 ± 1 ng/ml (protocol 2). Plasma insulin concentration increased (*P* < 0.05) during rhGH infusion (protocol 1) from 77 ± 22 to 141 ± 20 μU/ml. Insulin concentration did not change significantly after rhGH discontinuation (protocol 2) (from 131 ± 22 to 122 ± 33 μU/ml). Mean values of insulin concentrations were not significantly different in the pooled control studies (99 ± 20 μU/ml) and during rhGH infusions (136 ± 14 μU/ml; protocols 1 and 2). Plasma IGF-I concentration tended to increase (*P* = 0.08) during rhGH infusion (protocol 1) from 93 ± 14 to 153 ± 27 ng/ml. IGF-I concentration did not significantly change after rhGH discontinuation (protocol 2; from 188 ± 15 to 187 ± 17 ng/ml). Mean values of IGF-I concentrations were not significantly different in the pooled control studies (140 ± 21 ng/ml) and during rhGH infusions (170 ± 16 ng/ml; protocols 1 and 2).

Leg blood flow was similar in the control studies (5.25 ± 0.62 ml·min⁻¹·100 ml leg vol⁻¹) and during rhGH infusions (5.01 ± 0.83 ml·min⁻¹·100 ml leg vol⁻¹). The values of amino acid concentrations in femoral artery and vein were not significantly different in the control studies and during rhGH infusion (Table 1). In skeletal muscle, intracellular concentrations of most amino acids tended to decrease during rhGH infusion. Intramuscular glutamine concentrations decreased (*P* = 0.07) by ∼10%. Table 2 shows the values of amino acid balance across the leg in the control studies and during rhGH infusion. In the basal studies, the net balance of most amino acids was negative and significantly different from zero. Total amino acid release from leg muscle decreased after rhGH infusion by ∼55%. In particular, net phenylalanine, tyrosine, and lysine release, which are markers of net muscle protein catabolism, significantly decreased by ∼45–55% during rhGH infusion. Glutamine release also decreased significantly after rhGH infusion.

Table 3 shows the effects of rhGH infusion on selected parameters of muscle amino acid metabolism derived from leg arteriovenous balance of unlabeled amino acids (see Calculation of Kinetic Parameters). rhGH decreased the rates of net nonprotein glutamate

<table>
<thead>
<tr>
<th>Amino acid concentrations in femoral artery and vein and in leg muscle during saline and rhGH infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femoral Artery</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td><strong>Nonessential amino acids</strong></td>
</tr>
<tr>
<td>Glutamine</td>
</tr>
<tr>
<td>Glutamate</td>
</tr>
<tr>
<td>Alanine</td>
</tr>
<tr>
<td>Asparagine</td>
</tr>
<tr>
<td>Aspartate</td>
</tr>
<tr>
<td>Glycine</td>
</tr>
<tr>
<td>Serine</td>
</tr>
<tr>
<td>Tyrosine</td>
</tr>
<tr>
<td>Arginine</td>
</tr>
<tr>
<td>Histidine</td>
</tr>
<tr>
<td><strong>Essential amino acids</strong></td>
</tr>
<tr>
<td>Isoleucine</td>
</tr>
<tr>
<td>Leucine</td>
</tr>
<tr>
<td>Valine</td>
</tr>
<tr>
<td>Phenylalanine</td>
</tr>
<tr>
<td>Threonine</td>
</tr>
<tr>
<td>Lysine</td>
</tr>
</tbody>
</table>

Values are means ± SE. Units are nmol/ml whole blood or intracellular water. rhGH, recombinant human growth hormone.
Table 2. Leg balance of amino acids during saline and rhGH infusion

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Saline</th>
<th>rhGH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamine</td>
<td>−258 ± 44</td>
<td>144 ± 29†</td>
</tr>
<tr>
<td>Glutamate</td>
<td>131 ± 13</td>
<td>102 ± 15</td>
</tr>
<tr>
<td>Alanine</td>
<td>−221 ± 29</td>
<td>−200 ± 33</td>
</tr>
<tr>
<td>Asparagine</td>
<td>−32 ± 8‡</td>
<td>−22 ± 6†</td>
</tr>
<tr>
<td>Aspartate</td>
<td>−27 ± 9‡</td>
<td>−20 ± 13†</td>
</tr>
<tr>
<td>Glycine</td>
<td>−106 ± 7‡</td>
<td>−55 ± 16†</td>
</tr>
<tr>
<td>Serine</td>
<td>−18 ± 4‡</td>
<td>−4 ± 5</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>−34 ± 8‡</td>
<td>−14 ± 5‡</td>
</tr>
<tr>
<td>Arginine</td>
<td>−82 ± 17‡</td>
<td>12 ± 39</td>
</tr>
<tr>
<td>Histidine</td>
<td>−40 ± 9‡</td>
<td>−22 ± 7†</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>−14 ± 5‡</td>
<td>−7 ± 7</td>
</tr>
<tr>
<td>Leucine</td>
<td>7 ± 19</td>
<td>3 ± 12</td>
</tr>
<tr>
<td>Valine</td>
<td>−4 ± 18</td>
<td>−9 ± 11</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>−28 ± 4‡</td>
<td>−15 ± 3‡</td>
</tr>
<tr>
<td>Threonine</td>
<td>−79 ± 16‡</td>
<td>−51 ± 16†</td>
</tr>
<tr>
<td>Lysine</td>
<td>−82 ± 23‡</td>
<td>−45 ± 20†</td>
</tr>
<tr>
<td>Total amino acids</td>
<td>−854 ± 105</td>
<td>−389 ± 124</td>
</tr>
</tbody>
</table>

Values are means ± SE and are expressed as nmol · min⁻¹ · 100 ml leg vol⁻¹. Negative numbers indicate net release. *Significantly different from zero; †P < 0.05 rhGH vs. saline.

Table 3. Selected parameters of leg muscle amino acid metabolism during saline and rhGH infusion

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Saline</th>
<th>rhGH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Net alanine de novo synthesis</td>
<td>156 ± 28</td>
<td>165 ± 29</td>
</tr>
<tr>
<td>Net glutamine de novo synthesis</td>
<td>201 ± 43</td>
<td>113 ± 26†</td>
</tr>
<tr>
<td>Net nonprotein glutamate disposal</td>
<td>197 ± 20</td>
<td>139 ± 22</td>
</tr>
<tr>
<td>Total branched-chain amino acid disposal</td>
<td>196 ± 41</td>
<td>98 ± 20‡</td>
</tr>
</tbody>
</table>

Values are means ± SE and are expressed as nmol · min⁻¹ · 100 ml leg vol⁻¹. *P < 0.05 rhGH vs. saline.

Table 4. Amino acid enrichments in femoral artery and vein and in leg muscle during saline and rhGH infusion

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Femoral Artery</th>
<th>Femoral Vein</th>
<th>Leg Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylalanine</td>
<td>0.0381 ± 0.0021</td>
<td>0.0344 ± 0.0019</td>
<td>0.0248 ± 0.0025</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.0442 ± 0.0031*</td>
<td>0.0385 ± 0.0024*</td>
<td>0.0326 ± 0.0027*</td>
</tr>
<tr>
<td>Glutamine</td>
<td>0.0291 ± 0.0027</td>
<td>0.0236 ± 0.0015</td>
<td>0.0165 ± 0.0021</td>
</tr>
<tr>
<td>Total branched-chain amino acids</td>
<td>0.0535 ± 0.0043</td>
<td>0.0439 ± 0.0044†</td>
<td>0.0299 ± 0.0039</td>
</tr>
</tbody>
</table>

Values are means ± SE and are expressed as tracer/tracer ratio.
*P < 0.05 rhGH vs. saline.

rhGH almost doubled the rate of protein synthesis, whereas the hormone infusion did not significantly change the rate of protein degradation. Also, the rate of intracellular leucine release from protein degradation was not significantly different during saline (220 ± 40 nmol · min⁻¹ · 100 ml leg vol⁻¹) and rhGH (240 ± 42 nmol · min⁻¹ · 100 ml leg vol⁻¹) infusions. In addition, muscle mRNA levels of cathepsin B and UbB polyubiquitin did not significantly change after rhGH infusion (protocol 1; from 17 ± 2 to 19 ± 9% of GAPDH mRNA and from 4.9 ± 1.8 to 4.0 ± 0.2% of GAPDH mRNA, respectively) and after rhGH discontinuation (protocol 2; from 24 ± 9 to 28 ± 9% of GAPDH mRNA and from 10.0 ± 1.8 to 8.7 ± 3.4% of GAPDH mRNA, respectively).

Table 5 shows the rates of inward and outward transport of phenylalanine, leucine, and glutamine in skeletal muscle during saline and rhGH infusions. rhGH significantly increased the rates of inward transport of the essential amino acids leucine and phenylalanine, whereas the rates of glutamine transport were decreased by rhGH in both the outward and the inward directions. Figure 2 shows the isotopically derived rates of intramuscular glutamine de novo synthesis during saline and rhGH infusions. The absolute rate of glutamine de novo synthesis was 44 ± 11% lower during saline and rhGH infusions.
in skeletal muscle of hypercatabolic traumatized patients, 24-h rhGH infusion (protocol 1) increased muscle protein synthesis without significantly affecting protein degradation, 2) suppressed the rates of branched-chain amino acid catabolism, 3) increased the rates of transmembrane transport of the essential amino acids leucine and phenylalanine, whereas it decreased membrane transport of glutamine, 4) decreased the rates of de novo synthesis of glutamine, and 5) decreased the rates of release of total essential and nonessential amino acids from skeletal muscle.

Patients were studied during the second week after trauma in relatively stable clinical and metabolic conditions. They were hypercatabolic, as shown by the net efflux of essential amino acids from skeletal muscle despite a continuous combined parenteral and enteral artificial nutrition. We have shown that rhGH administration significantly reduces the net muscle protein loss of patients. This anabolic effect of rhGH is completely accounted for by an acceleration of protein synthesis. Previous studies have demonstrated a selective increase in muscle protein synthesis induced by rhGH administration in humans (12, 13). It is possible, however, that many of the observed anabolic effects of rhGH are mediated via the stimulation of endogenous IGF-I synthesis, which may exert its effects via endocrine and/or paracrine mechanisms. In humans, IGF-I administration promoted protein anabolism both by stimulating protein synthesis and by inhibiting protein degradation both in muscle and at the whole body level (10, 11). In our study, rhGH administration did not result in significant modifications of the rates of muscle protein degradation. The effects of rhGH on protein degradation were evaluated with different approaches. Stable isotopes and mass spectrometry techniques were used to measure the rate of protein degradation in skeletal muscle as the rate of appearance of the essential amino acids phenylalanine and leucine in muscle cells.

With competitive PCR, we determined in skeletal muscle the mRNA levels of cathepsin B and ubiquitin as markers of nonmyofibrillar and myofibrillar protein degradation, respectively. It is known that growth hormone has the potential to decrease proteolysis, possibly through stimulation of IGF-I synthesis (10, 11). In this study, we did not observe any decrease in muscle proteolysis: we observed a substantial stimulation of protein synthesis and a tendency toward decreasing intracellular concentration of total amino acids. It is likely that the rate of degradation was maintained and possibly increased to prevent a further decrease in amino acid concentrations.

In contrast to the rhGH effects on muscle proteolysis, at the whole body level, the rate of phenylalanine appearance was significantly lower during the hormone infusion. Such a decrease of a marker of whole body proteolysis clearly indicates a systemic effect of the intra-arterial rhGH infusion. In fact, in the systemic circulation, not only growth hormone but also insulin and IGF-I increased during the local rhGH infusion. It is likely that such a decrease of whole body proteolysis could have been mediated by insulin and/or IGF-I. Furthermore, such systemic hormonal changes could also have contributed to the stimulation of muscle protein synthesis observed in our study (3, 6, 11).

In agreement with previous observations in animals (20, 23), this study shows that rhGH infusion in traumatized patients accelerates the rates of transmembrane transport of the essential amino acids leucine and phenylalanine. This effect was independent of changes of leg blood flow and arterial amino acid concentrations. This rhGH-mediated increased ability of transmembrane systems to transport essential amino acids confirms previous observations in vitro (20, 23) and represents a novel observation in vivo. This acceleration of transport of essential amino acids may have contributed to the anabolic effect of rhGH by increasing intracellular amino acid availability for protein synthesis. However, the slight decrease in intracellular amino acid concentrations suggests that the stimulation of amino acid transport is not the primary mechanism for stimulation of synthesis. In contrast, transport acceleration may represent a compensatory

### Table 5. Transmembrane amino acid transport

<table>
<thead>
<tr>
<th></th>
<th>Phenylalanine</th>
<th>Leucine</th>
<th>Glutamine</th>
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<tbody>
<tr>
<td><strong>Inward transport</strong></td>
<td></td>
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<tr>
<td>Saline</td>
<td>119 ± 20</td>
<td>303 ± 68</td>
<td>1316 ± 276</td>
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<tr>
<td>rhGH</td>
<td>228 ± 53*</td>
<td>558 ± 117*</td>
<td>848 ± 170*</td>
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<tr>
<td><strong>Outward transport</strong></td>
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<tr>
<td>Saline</td>
<td>147 ± 19</td>
<td>296 ± 73</td>
<td>1574 ± 314</td>
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<tr>
<td>rhGH</td>
<td>243 ± 53</td>
<td>556 ± 122*</td>
<td>992 ± 192*</td>
</tr>
</tbody>
</table>

Values are means ± SE and are expressed as nmol·min⁻¹·100 ml leg vol⁻¹. *P < 0.05 rhGH vs. saline.

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**DISCUSSION**

During rhGH infusion, the rates of branched-chain amino acid catabolism were significantly increased (P = 0.03) (20, 23). This effect was independent of the systemic changes of leg blood flow and arterial amino acid concentrations. This rhGH-mediated increased ability of transmembrane systems to transport essential amino acids confirms previous observations in vitro (20, 23) and represents a novel observation in vivo. This acceleration of transport of essential amino acids may have contributed to the anabolic effect of rhGH by increasing intracellular amino acid availability for protein synthesis. However, the slight decrease in intracellular amino acid concentrations suggests that the stimulation of amino acid transport is not the primary mechanism for stimulation of synthesis. In contrast, transport acceleration may represent a compensatory

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**Fig. 2.** Absolute rate of de novo glutamine synthesis in leg skeletal muscle of trauma patients during saline or rhGH infusion. *P < 0.05 rhGH vs. saline.
mechanism to prevent depletion of intracellular amino acid pools.

Besides stimulating protein synthesis, growth hormone suppressed the rate of catabolism of the branched-chain amino acids leucine, isoleucine, and valine. This effect has been reported by several other authors using isotopic tracers of leucine at the whole body level (8, 12).

In this study, the anticatabolic growth hormone effects on protein and branched-chain amino acid metabolism were paralleled by a suppression of the rate of de novo muscle glutamine production. De novo muscle glutamine production was determined either as abso-

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between the extra- and intracellular spaces in muscle samples. This value had been obtained previously in normal human subjects (2). Nonetheless, despite the fact that none of our patients showed clinical evidence of changes in fluid retention during the study, we cannot exclude the possibility that growth hormone could have slightly increased extracellular space volume in muscle tissue. In fact, sodium and water retention are well recognized side effects of rhGH administration (16). We therefore evaluated the potential effects of changes in the ratio between the extra- and intracellular compartments on the calculated kinetic parameters of protein and glutamine metabolism. When such a ratio was increased by 100% over the assumed initial value of 0.16, the changes in protein synthesis and degradation, glutamine de novo synthesis, and amino acid transport were <5%. It is unlikely, therefore, that growth hormone-mediated changes in extracellular fluid in muscle could have affected the conclusions of our study.

Takala et al. (31) recently reported the results of two large multicenter studies indicating an increased morbidity and mortality in critically ill patients treated with high doses of growth hormone. Multiple organ failure and septic shock or uncontrolled infections were the main causes of death, suggesting that a modulation of the immune system or gut function was involved. However, the reason for such a deleterious effect of growth hormone is unclear. Our study describes a potential side effect of rhGH administration in critically ill patients. In these patients, glutamine is an essential substrate for rapidly dividing cells in the immune system and in the intestinal mucosa, as well as for glutathione synthesis in the liver. Skeletal muscle is the main tissue involved in glutamine de novo synthesis. In our patients, whole body skeletal muscle released ~19 g of glutamine per day into the bloodstream before rhGH administration [to extrapolate the data to whole body skeletal muscle (33), the data for one leg were multiplied by four]. After rhGH administration, glutamine release from skeletal muscle decreased by ~50%, whereas at the whole body level, glutamine clearance tended to decrease by ~15%, despite the fact that the highest growth in hormone levels was achieved in only one leg. We may speculate, therefore, that in a clinical setting, rhGH therapy could decrease systemic glutamine availability and have negative effects on the immune system and gut function. The obvious solution for this potential side effect of growth hormone treatment in critically ill patients is to simultaneously administer exogenous glutamine to offset the decreased availability of the endogenous amino acid.

We thank Prof. F.E, Baralle and Prof. M. Giacca (International Center for Genetic Engineering and Biotechnology, Trieste), and Dr. R. Situlin (Istituto di Clinica Medica, University of Trieste) for invaluable advice and collaboration. We thank Drs. L. Luizi and A. Battezzati (San Raffaele Scientific Institute, Milan) for the analysis of IGF-I plasma levels. The human recombinant growth hormone and additional support were kindly provided by Pharmacia and Upjohn (Milan, Italy). We also wish to express our appreciation to Anna De Santis and Mariella Sturma for excellent technical assistance.

G. Biolo was awarded the European Society of Parenteral and Enteral Nutrition (ESPEN)-Clintec Fellowship 1994 (“Effects of growth hormone administration on skeletal muscle glutamine metabolism in severely traumatized patients”). A. Bosutti was awarded the ESPEN Fellowship 1996 (“Molecular regulation of protein catabolism in trauma patients”).

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