Inhibition of muscle glutamine formation in hypercatabolic patients

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ABSTRACT

Glutamine is synthesized primarily in skeletal muscle, and enables transfer of nitrogen to the liver, as well as serving other functions. There is increasing evidence for beneficial clinical effects of glutamine supplementation in critically ill patients. However, the response of endogenous glutamine formation to severe stress is poorly understood. The rates of net protein balance, leucine oxidative decarboxylation, and alanine and glutamine synthesis de novo were determined in leg skeletal muscle of 20 severely burned patients and 19 normal controls in the post-absorptive state. Patients were studied at 14±5 days post-burn, and their mean burn size was 66±18% of total body surface area. Methods were based on the leg arteriovenous balance technique in combination with biopsies of the vastus lateralis muscle. In the post-absorptive state, patients with severe burns, as compared with healthy control subjects, exhibited accelerated muscle loss (>150%) (i.e. proteolysis minus synthesis) and leucine oxidative decarboxylation (>117%), and depletion of the intramuscular free glutamine pool (<63%). The average rate of glutamine synthesis de novo was decreased by 48%, whereas net alanine synthesis de novo was increased by 174%, in skeletal muscle of burned patients. In conclusion, in severely hypercatabolic burned patients, muscle glutamine formation was suppressed, whereas alanine was the major vehicle for inter-organ nitrogen transport. These changes account for a decreased glutamine availability during prolonged severe stress.

INTRODUCTION

Glutamine is a non-essential amino acid that is synthesized primarily in skeletal muscle and released into the bloodstream. In tissues other than muscle, glutamine is a precursor of many compounds, including hepatic glucose, urinary ammonia, intracellular glutathione (through glutamate) and nucleic acids. Furthermore, glutamine is a major fuel for rapidly dividing cells of the intestinal mucosa and immune system [1]. There is increasing evidence for beneficial metabolic and clinical effects of glutamine supplementation in critically ill patients [2–6]. During stress, glutamine has been considered to be a ‘conditionally’ essential amino acid [7]. This view is mainly based on the increase in glutamine uptake in certain tissues (e.g. kidneys, intestinal mucosa and immune cells) and the dramatic decline in muscle free glutamine which have been consistently observed in trauma and infections [8]. Nonetheless, the ability of muscle tissue to provide glutamine for peripheral tissues in severe stress is poorly understood. In the present study we have determined the rate of glutamine synthesis de novo in skeletal muscle in the post-absorptive state in normal controls and in patients with severe burns during the ‘flow’ phase after injury.

METHODS

Patients
We studied 19 normal volunteers and 20 patients with severe burns in the post-absorptive state. The volunteers [18 males and one female; age 29±10 years (mean ±
S.D.); body mass index 24 ± 3 kg/m²) were admitted to the General Clinical Research Center at the University of Texas Medical Branch on the morning of the study. The patients with burns (17 males and three females; age 31 ± 15 years; body mass index 24 ± 5 kg/m²) were studied 8–30 days (mean ± S.D. 14 ± 4 days) after injury. The proportion of body surface burned ranged from 40% to 90% (mean ± S.D. 66 ± 18%). The extent of third-degree burns was 55 ± 30% of total body surface area. The patients were admitted either to the University of Texas Medical Branch (n = 16) or to the Shriners Burns Hospital in Galveston (n = 4) within 48 h after injury. Fluid resuscitation had been provided, and excision of the burn wound and grafting had been carried out within 4 days after the injury, as previously described [9]. Our studies were performed at least 2 days after the most recent surgical excision. The patients were studied during a relatively stable clinical and metabolic period, when they were ascertained to be in the phase of response generally characterized by elevations in cardiac rate (130 ± 23 beats/min), core temperature (38.4 ± 0.4 °C) and metabolic rate (25 ± 7% increase in resting energy expenditure above the expected values calculated using the Harris–Benedict equation). All patients received continuous enteral feeding of milk via a duodenal feeding tube at a rate to provide 7531 kJ/m² (1800 kcal/m²) of body surface area per day plus 9205 kJ/m² (2200 kcal/m²) of body surface area burned per day. All forms of nutritional support were stopped 8 h before the start of isotope infusion. All normal volunteers, patients or patients’ parents gave informed written consent before participating in the study, which was approved by the Institutional Review Board of the University of Texas Medical Branch at Galveston, Texas.

**Experimental protocol**

The study was designed to determine, in the post-absorptive state, the leg arteriovenous balance of glucose and of selected amino acids, and to evaluate steady-state conditions of intracellular free amino acid concentrations in leg muscle. Indwelling catheters were placed in the femoral artery and vein of one leg for blood sampling. The femoral arterial catheter was also used for the primed/continuous infusion of Indocyanine Green (Becton Dickinson Microbiology Systems, Cockeysville, MA, U.S.A.) in order to measure leg blood flow [10]. Indocyanine Green recycling was assessed by measuring dye concentration in an antecubital vein. In each subject, two muscle biopsies were taken in the post-absorptive state at time 0 (i.e. around 08.00 hours) and after 3 h from the lateral portion of the vastus lateralis muscle. In order to measure leg blood flow, at 1 h and 45 min a primed (5 mg)/continuous (0.5 mg/min) infusion of Indocyanine Green dye was started into the femoral artery, which was maintained until the end of the experiment. Between 2 h and 3 h, blood samples were taken every 20 min from the femoral and antecubital veins and the femoral artery. To allow sampling from the femoral artery, the dye infusion was stopped for less than 10 s and then quickly resumed [10]. Arterial samples were always taken after samples from the femoral and wrist veins, to avoid interference with the blood flow measurements.

**Analysis**

Concentrations of glucose, phenylalanine, leucine, alanine and glutamine were measured in whole-blood samples from the femoral artery and vein and in muscle tissue, using an internal standard technique and GC/MS analysis (Hewlett-Packard 5985) [10]. Measured values of concentrations in muscle tissue were corrected for total tissue water, as described in [10], to obtain intracellular values. Leg plasma flow was calculated from steady-state values of dye concentrations in the femoral and antecubital veins [10]. Leg blood flow was calculated from the haematocrit and plasma flow.

**Calculations and statistical analysis**

The net balance in the leg for glucose and amino acids was calculated from the Fick principle:

\[
\text{Net balance} = (C_A - C_V) \times \text{leg blood flow}
\]

where \(C_A\) and \(C_V\) are the whole-blood glucose or amino acid concentrations in the femoral artery and vein respectively. Negative values indicate net release, whereas positive values indicate net uptake. Skeletal muscle is considered to largely account for amino acid and glucose metabolism in the whole leg [10]. In steady-state conditions for amino acid concentrations in muscle tissue and in the femoral artery and vein, amino acid uptake or release across the leg reflects the balance between intracellular amino acid disposal and production for that particular amino acid. Since phenylalanine is neither synthesized nor oxidized in muscle tissue [11], phenylalanine release from leg muscle is a marker of muscle protein balance (i.e. proteolysis minus synthesis) [10]. In contrast, skeletal muscle is the main site of oxidative deamination of the branched-chain amino acid leucine [11] and of the synthesis de novo of alanine and glutamine [10]. We have assumed that amino acids are released from proteolysis in proportion to their relative content in muscle protein [10]. Thus the rates of release from net protein degradation of glutamine, alanine and leucine can be calculated from the rate of net phenylalanine balance corrected for the molar ratios of leucine/phenylalanine (i.e. 3.10), alanine/phenylalanine (i.e. 2.35) and glutamine/phenylalanine (i.e. 2.05) determined in mixed human muscle protein [10]. Then the rates of net alanine and glutamine synthesis and appearance in the bloodstream and of leucine catabolism can be calculated by subtracting from the net balance of these amino acids...
the component accounted for by net protein degradation. Thus [10,12]:

Leucine oxidative decarboxylation = (leucine net balance) – (phenylalanine net balance × 3.10);
Net alanine synthesis de novo = (alanine net balance) – (phenylalanine net balance × 2.35);
Net glutamine synthesis de novo = (glutamine net balance) – (phenylalanine net balance × 2.05).

Data are expressed as means ± S.D. The values in the patient group were compared with those in the control group by means of Student’s t-test for non-paired samples.

RESULTS

Concentrations of amino acids and glucose in the femoral artery and vein, as well as of amino acids in muscle tissue, were constant during the study period. Their mean values are reported in Table 1. In the patients with burns, intracellular glutamine concentrations in skeletal muscle were about 40% of that in the controls, whereas phenylalanine, leucine and alanine concentrations were higher (Table 1). Leg blood flow was twice as high (P < 0.001) in the patients than in the control subjects (7.66 ± 2.14 and 3.47 ± 1.38 ml·min⁻¹·100 ml⁻¹ leg volume respectively).

The patients were in a hypercatabolic state, as indicated by their significantly higher rate of net phenylalanine release from muscle protein degradation in the post-absorptive state when compared with that in the normal volunteers (Table 2). Leucine and alanine release from leg muscle were greater in the patients with burns than in the control subjects. In contrast, the rate of muscle glutamine release tended to be lower in burned patients than in the controls. Glucose uptake was greater in the patients than in the control group. The rate of oxidative decarboxylation of leucine was significantly greater (P < 0.001) in the patients with burns (69 ± 37 nmol·min⁻¹·100 ml⁻¹ leg volume) than in the control subjects (32 ± 20 nmol·min⁻¹·100 ml⁻¹ leg volume).

Table 1  Amino acid and glucose concentrations in whole blood and skeletal muscle in burned patients and control subjects

<table>
<thead>
<tr>
<th>Site</th>
<th>Phenylalanine (nmol/ml)</th>
<th>Leucine (nmol/ml)</th>
<th>Alanine (nmol/ml)</th>
<th>Glutamine (nmol/ml)</th>
<th>Glucose (µmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femoral artery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>48 ± 10</td>
<td>11 ± 23</td>
<td>249 ± 79</td>
<td>549 ± 71</td>
<td>5.52 ± 0.12</td>
</tr>
<tr>
<td>Burns</td>
<td>68 ± 14*</td>
<td>124 ± 26</td>
<td>230 ± 85</td>
<td>422 ± 74*</td>
<td>5.46 ± 0.48</td>
</tr>
<tr>
<td>Femoral vein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>53 ± 11</td>
<td>124 ± 26</td>
<td>287 ± 87</td>
<td>615 ± 55</td>
<td>5.40 ± 0.12</td>
</tr>
<tr>
<td>Burns</td>
<td>74 ± 15*</td>
<td>134 ± 27</td>
<td>276 ± 97</td>
<td>447 ± 76*</td>
<td>5.34 ± 0.48</td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>91 ± 25</td>
<td>207 ± 36</td>
<td>1676 ± 416</td>
<td>12,132 ± 2440</td>
<td>–</td>
</tr>
<tr>
<td>Burns</td>
<td>139 ± 51*</td>
<td>373 ± 163*</td>
<td>2052 ± 743</td>
<td>4,469 ± 1709*</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 2  Leg amino acid and glucose balance in burned patients and control subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>Phenylalanine balance (nmol·min⁻¹·100 ml⁻¹ leg volume)</th>
<th>Leucine balance (nmol·min⁻¹·100 ml⁻¹ leg volume)</th>
<th>Alanine balance (nmol·min⁻¹·100 ml⁻¹ leg volume)</th>
<th>Glutamine balance (nmol·min⁻¹·100 ml⁻¹ leg volume)</th>
<th>Glucose balance (µmol.ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>– 18 ± 8</td>
<td>– 24 ± 21</td>
<td>– 124 ± 57</td>
<td>– 228 ± 80</td>
<td>438 ± 186</td>
</tr>
<tr>
<td>Burns</td>
<td>– 45 ± 20*</td>
<td>– 72 ± 40*</td>
<td>– 331 ± 146*</td>
<td>– 193 ± 52</td>
<td>924 ± 270*</td>
</tr>
</tbody>
</table>
Calculated values of alanine and glutamine synthesis de novo are shown in Figure 1. In the patients with burn injuries, the average rate of muscle glutamine synthesis de novo was 48% lower than in the normal controls. On the other hand, the average rate of alanine synthesis de novo was 174% higher in the patients than in the normal controls.

**DISCUSSION**

Patients with severe burn injury were studied during the flow phase under conditions of accelerated protein and amino acid catabolism. These patients also expressed the characteristic depletion of the intramuscular and circulating glutamine pools. We found that, in muscle, the decrease in glutamine concentration was paralleled by a suppression of the rate of net synthesis of this amino acid. Consequently, systemic glutamine availability from muscle synthesis de novo was decreased by about 48% in the patients with burns.

In contrast with glutamine, muscle alanine synthesis de novo was increased by 174% in the patients with burns. These results demonstrate, therefore, that alanine constitutes the major carrier of nitrogen from skeletal muscle to body tissues in conditions of prolonged severe stress. Our results and previous evidence show that, after burn injury, the rates of glucose uptake [13], glycolysis [14] and oxidation of branched-chain amino acids [15] are increased. This leads to an increase in the availability of pyruvate and of amino-nitrogen for transamination. As a result, pyruvate is transaminated to alanine at rates that are greater than normal [14]. This mechanism potentially limits the availability of glutamate as a precursor for the synthesis de novo of glutamine through increased transamination to form alanine (Figure 2). Several lines of evidence support this hypothesis. First, levels of glutamate are decreased in trauma patients [16], and supplementation with 2-oxoglutarate (α-ketoglutarate) preserved muscle free glutamine levels after surgery [17,18]. Secondly, stimulation of pyruvate oxidation by dichloroacetate administration decreases alanine production [14] and increases the muscle glutamine concentration [19] in patients with burns. Finally, the muscle free ammonia concentration is elevated in severe stress [20]. Therefore the reduced glutamine formation in severe burn injury may be the result of a depletion of glutamate, the immediate glutamine precursor, which is preferentially consumed for alanine synthesis. The fact that the activity of the enzyme glutamine synthetase has often been found to be increased in catabolic conditions [21] is consistent with the notion that, whereas the muscle is capable of producing glutamine at an accelerated rate, in the post-absorptive state there is an inadequate supply of precursors.

In normal subjects, the arteriovenous balance of amino acids across a leg is largely representative of skeletal muscle metabolism, and the contribution of normal skin is negligible [10]. To determine whether the presence of a leg burn wound may significantly contribute to leg metabolism, we have compared results in patients with (n = 14; total leg surface burned 64 ± 24%; total body surface burned 70 ± 19%) and without (n = 6; total body surface burned 59 ± 12%) leg burn. In the patients with and without leg burns, phenylalanine release from proteolysis was 44 ± 20 and 49 ± 20 nmol of phenylalanine min⁻¹ 100 ml⁻¹ leg volume respectively; leucine catabolism was 74 ± 32 and 57 ± 21 nmol min⁻¹ 100 ml⁻¹ leg volume respectively; alanine synthesis was 227 ± 20 and 193 ± 47 nmol min⁻¹ 100 ml⁻¹ leg volume respectively; and glutamine synthesis was 102 ± 47 and 83 ± 32 nmol min⁻¹ 100 ml⁻¹100 ml leg volume respectively. Thus, in agreement with previous preliminary observations [22], our data indicate that the determination of muscle protein and amino acid metabolism is not significantly affected by the presence of a burn wound in the catheterized leg.

The results of the present study rely on the assumption that amino acids are released from protein degradation in proportion to their relative content in muscle protein. Phenylalanine is used as a marker of protein balance (synthesis minus breakdown), since this amino acid is neither synthesized nor oxidized in muscle tissue. We have determined the amino acid content in human muscle protein by acidic hydrolysis, GC/MS, and stable isotopes as internal standard [10]. We measured the sum of glutamine and glutamate using the derivative that makes the two amino acids undistinguishable by GC/MS analysis [10]. The glutamine content was then calculated by assuming a glutamine/glutamate molar ratio of 0.56, as reported by Kominz et al. [23] and Ruderman and Berger [24] for muscle protein. A recent determination of glutamine content in muscle protein via pre-hydrolysis derivatization suggested a glutamine/glutamate molar
Glutamine synthesis in burn patients

Figure 2 Relationship between glutamine and alanine synthesis, branched-chain amino acid oxidation, glucose utilization and protein catabolism in skeletal muscle of severely burned patients

Some of the metabolic pathways represented were directly assessed in the present study. For protein catabolism, the arrow indicates net protein balance, i.e. the difference between proteolysis and synthesis. Bold solid arrows indicate metabolic pathways found to be stimulated in the patients. Bold broken arrows indicate metabolic pathways found to be inhibited in the patients.

The ratio of approx. 0.35 [25], which would imply a glutamine/phenylalanine molar ratio of 0.92. When such a ratio was used in the present study, the calculated rate of glutamine synthesis de novo increased, but remained significantly lower \((P = 0.02)\) in the burned patients \((151 \pm 49 \text{ nmol} \cdot \text{min}^{-1} \cdot 100 \text{ ml}^{-1} \text{ leg volume})\) as compared with the control subjects \((212 \pm 81 \text{ nmol} \cdot \text{min}^{-1} \cdot 100 \text{ ml}^{-1} \text{ leg volume})\).

In a recent paper [26] we reported that, in a small group of patients \((n = 5)\) similar to those of the present study (but studied in the fed state), there was a several-fold increase in the rate of intramuscular glutamine catabolism, and that although the rate of glutamine synthesis was elevated, the intracellular glutamine concentration was decreased. Those conclusions were based on an isotopic technique not used in the present experiment. In the present paper, only the balance between glutamine synthesis and glutamine degradation was determined (i.e. net glutamine production). ‘Net’ glutamine production was not the focus of the other paper [26], but the value of ‘net’ glutamine production in that paper was almost identical to the value reported in the present paper. This similarity in results is striking, given that the previous small group of patients were studied in the fed state, whereas in the present study the patients had fasted.

Thus, regardless of changes in intracellular glutamine kinetics, net glutamine production from muscle is decreased in severely burned patients, whether studied in the fed or fasted state.

In conclusion, we have found that there is a suppression of endogenous ‘net’ glutamine formation in skeletal muscle of patients with severe burns. The present study supports the concept that, in severe stress situations, glutamine becomes a conditionally essential amino acid, and provides the rationale for glutamine supplementation in trauma patients [4].

ACKNOWLEDGMENTS

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