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PRELIMINARY REPORT

Contribution of the Ubiquitin-Proteasome Pathway to Overall Muscle Proteolysis in Hypercatabolic Patients

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The influence of the gene expression of critical components of the cytoplasmic and lysosomal proteolytic pathways on the rate of protein degradation was evaluated in the leg skeletal muscle of 8 severely traumatized patients. Muscle proteolysis was determined as the intramuscular phenylalanine rate of appearance by L-[ring-$^2H_5$]phenylalanine infusion and the leg arteriovenous catheterization technique combined with muscle biopsy. Muscle mRNA levels of UbB polyubiquitin and cathepsin B were determined by reverse transcriptase–competitive polymerase chain reaction and expressed as a percent of the mRNA level of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). In the patients, individual values for UbB polyubiquitin mRNA levels directly correlated with the rate of muscle proteolysis ($r = .76$, $P < .05$), whereas no correlation ($r = .10$) was found between cathepsin B mRNA levels and proteolysis. Thus, after trauma, the rate of muscle proteolysis appears to be largely regulated by the ubiquitin-proteasome system at the level of gene transcription.

PATIENTS WITH SEVERE INJURIES are characterized by a persistent loss of skeletal muscle protein, which may have a very negative impact on survival and rehabilitation. A sustained increase in proteolysis is the major determinant of muscle atrophy in these patients.[1] Muscle proteins can be degraded by different proteolytic pathways. In the lysosomes, cathepsin B, D, E, H, and L are mainly involved in the degradation of nonmyofibrillar proteins such as membrane-associated proteins and endocytosed proteins. In the cytoplasm, the ubiquitin-proteasome system degrades the bulk of myofibrillar protein. In patients with severe trauma, gene expression for the components of both the ubiquitin-proteasome and cathepsin systems appears to be increased.[2] However, the relative importance of these two proteolytic pathways in the regulation of muscle proteolysis after trauma is not completely understood.

To this aim, we have simultaneously determined the rate of overall muscle protein degradation[3] and the mRNA levels of cathepsin B and UbB polyubiquitin,[4,5] critical components of the cathepsin and ubiquitin systems, in the skeletal muscle of severely traumatized patients.

SUBJECTS AND METHODS

Eight adult male patients with multiple injuries (APACHE II score, 15 ± 3) were studied 8 to 12 days after trauma, ie, during the late flow-phase postinjury characterized by a relatively stable clinical condition despite an increased energy requirement and protein catabolism. Informed consent was obtained from the patients' close relatives. The protocol was approved by the competent Hospital Authority. All patients received continuous combined intravenous and enteral nutrition (20% of total calories). None of the patients had injuries involving the leg on which determinations of muscle protein kinetics and protease mRNA levels were made.

Leg muscle protein degradation was determined as the rate of intracellular phenylalanine appearance by the isotope dilution technique as previously described.[3] During primed-continuous infusions of L-[ring-$^2H_5$]phenylalanine (Mass Trace, Woburn, MA), blood samples from the femoral artery and vein and muscle biopsies from the vastus lateralis muscle were taken to measure steady-state values of the phenylalanine concentration and enrichment by high-performance liquid chromatography (Beckman, Berkeley, CA) and gas chromatography/mass spectrometry (Finnigan MAT, Bremen, Germany), respectively. Leg blood flow was measured by the dye-dilution technique using indocyanine green (Infracyanine; SERB, Paris, France). Calculations are described in our previous report.[3] Muscle mRNA levels of UbB polyubiquitin and

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cathepsin B were determined as previously described[4] by reverse transcriptase–competitive polymerase chain reaction (Perkin Elmer, Norwalk, CT) and are expressed as a percentage of the mRNA level of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH).[4,5] It is assumed that GAPDH mRNA levels remain constant in different conditions.[2] Ubiquitin is encoded in the human genome as a multigene family.[6] Among the different ubiquitin genes, we have assessed the UbB polyubiquitin gene that codes for 3 direct repeats of the ubiquitin sequence.[6]

RESULTS

The patients with injuries were in a hypercatabolic state, as indicated by the net phenylalanine release from the leg muscle (31 ± 5 nmol phenylalanine/min/100 mL leg vol) despite optimized continuous nutritional support. Figure 1 shows that individual values for muscle UbB polyubiquitin mRNA levels directly correlated ($r = .76$, $P = .03$) with the rate of protein degradation, whereas no correlation ($r = .10$) was found between cathepsin B mRNA and protein degradation (Fig 2). Also, multiple linear regression analysis (for muscle proteolysis as the dependent variable and UbB polyubiquitin and cathepsin B mRNA as independent variables) did not indicate any influence of cathepsin B mRNA on muscle protein degradation.

DISCUSSION

Patients with severe trauma experience a rapid loss of muscle protein, primarily due to enhanced proteolysis. In this study, we have simultaneously determined the rate of proteolysis using isotopic amino acid tracers, and the gene expression of critical components of the cytoplasmic (ie, ubiquitin-proteasome pathway) and lysosomal (ie, cathepsin system) proteases in skeletal muscle of hypercatabolic trauma patients. The results indicate that after trauma, the rate of muscle proteolysis is largely regulated by the ubiquitin-proteasome system at the level of gene transcription, as illustrated by the direct relationship ($r = .76$) between UbB polyubiquitin mRNA levels and the rate of phenylalanine appearance in skeletal muscle cells (Fig 1). In contrast, we found no correlation ($r = .10$) between cathepsin B mRNA levels and the rate of proteolysis. This suggests a minor role of the cathepsin system in the regulation of muscle protein kinetics in hypercatabolic conditions.

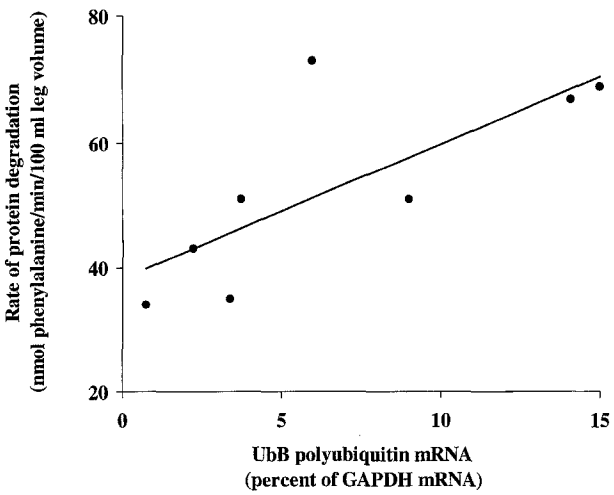

Fig 1. Relationship between individual values for UbB polyubiquitin mRNA level and isotopically determined rate of protein degradation in skeletal muscle of severely traumatized patients ($r = .76$, $P = .03$).

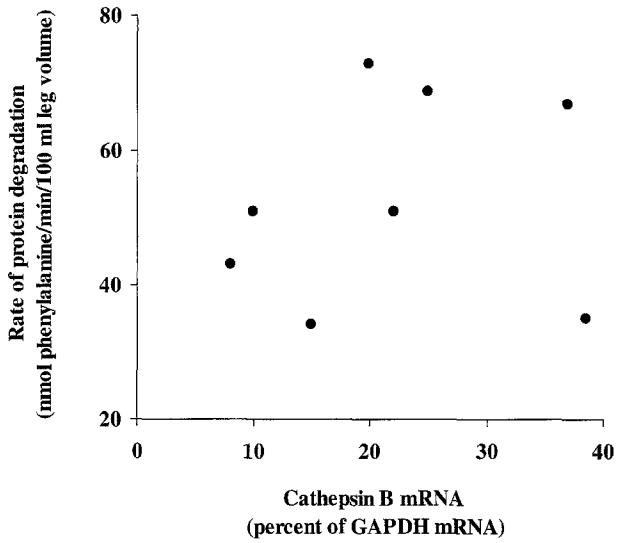

Fig 2. Relationship between individual values for cathepsin B mRNA level and isotopically determined rate of protein degradation in skeletal muscle of severely traumatized patients ($r = .10$).

These data corroborate previous studies that demonstrated parallel increases of proteolysis and of mRNAs encoding ubiquitin and proteasome subunits under different hypercatabolic conditions such as trauma, sepsis, and metabolic acidosis.[1,2,7] Furthermore, in our study, the lack of correlation between cathepsin B mRNA levels and muscle proteolysis is supported by previous evidence showing that the lysosomal pathway is not quantitatively important in the turnover of muscle proteins[8] and does not change consistently in critical illness.[7,9,10] Cathepsins are involved in the degradation of nonmyofibrillar proteins and are regulated by nutrient availability (especially amino acids) and insulin levels.[1,9,10] Our patients were studied 8 to 12 days after injury, in a relatively stable clinical condition, during continuous combined intravenous and enteral nutrition providing 250 mg nitrogen/kg/d. Their plasma insulin was at a level typically found in the postprandial state, ie, about 80 μU/mL. It is conceivable that such optimized nutritional support may have blunted the component of overall muscle proteolysis accounted for by the lysosomal pathway. However, we cannot exclude that the cathepsin system may play a role in the muscle catabolic response to pathological conditions other than trauma, during insufficient nutritional support or during an earlier phase postinjury.

In conclusion, our results suggest that in hypercatabolic trauma patients, the therapy for excessive muscle proteolysis should be targeted at modulation of the ubiquitin-proteosome system.[1]

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