Proteasome proteolytic activity in skeletal muscle is increased in patients with sepsis

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ABSTRACT

Patients with sepsis in the ICU (intensive care unit) are characterized by skeletal muscle wasting. This leads to muscle dysfunction that also influences the respiratory capacity, resulting in prolonged mechanical ventilation. Catabolic conditions are associated with a general activation of the ubiquitin-proteasome pathway in skeletal muscle. The aim of the present study was to measure the proteasome proteolytic activity in both respiratory and leg muscles from ICU patients with sepsis and, in addition, to assess the variation of proteasome activity between individuals and between duplicate leg muscle biopsy specimens. When compared with a control group (n = 10), patients with sepsis (n = 10) had a 30 % (P < 0.05) and 45 % (P < 0.05) higher proteasome activity in the respiratory and leg muscles respectively. In a second experiment, ICU patients with sepsis (n = 17) had a 55 % (P < 0.01) higher proteasome activity in the leg muscle compared with a control group (n = 10). The inter-individual scatter of proteasome activity was larger between the patients with sepsis than the controls. We also observed a substantial intra-individual difference in activity between duplicate biopsies in several of the subjects. In conclusion, the proteolytic activity of the proteasome was higher in skeletal muscle from patients with sepsis and multiple organ failure compared with healthy controls. It was shown for the first time that respiratory and leg muscles were affected similarly. Furthermore, the variation in proteasome activity between individuals was more pronounced in the ICU patients for both muscle types, whereas the intra-individual variation between biopsies was similar for ICU patients and controls.

INTRODUCTION

Patients with septic shock in the ICU (intensive care unit) are prone to develop muscle wasting, a condition that is mainly manifested in skeletal muscle. The net loss of muscle proteins could depend on decreased protein synthesis, an increase in protein degradation or a combination of both. Despite the progressive loss of muscle proteins during critical illness, fractional protein synthesis rate in skeletal muscle is on average within the range of healthy subjects [1–3]. This indicates that muscle wasting in these patients is mainly caused by an increase in protein degradation.

The majority of intracellular proteins, including myofibrillar proteins in muscle, are degraded via the ubiquitin-proteasome pathway [4]. Most proteins degraded...
by this pathway are first marked with a polyubiquitin chain in order to be degraded by the 26 S proteasome. The 26 S proteasome consists of two 19 S regulatory domains and a central 20 S protease core. The proteolytic activity comprises two chymotrypsin-like, two trypsin-like and two caspase-like active sites. The chymotrypsin-like activity is the rate-limiting step and initially cleaves the protein, thereby stimulating the caspase-like activity [5]. Catabolic conditions have been associated with an increase in mRNA expression of several subunits of this pathway, as well as an increase in the proteasome proteolytic activity. Increased levels of mRNA encoding ubiquitin and subunits of the 20 S proteasome have been found in animal models of muscle wasting caused by fasting and denervation [6], sepsis [7], diabetes [8] and glucocorticoid treatment [9]. Also, studies of patients with different catabolic diseases, such as trauma [10,11], cancer [12,13] and sepsis [14], have revealed increased mRNA levels of ubiquitin and proteasome subunits. The proteolytic activity of the proteasome can be studied in vitro in muscle extracts incubated with fluorogenic peptide substrates. In animal experiments, increased activities were found after induction of sepsis or burn injury [7,15]. In addition, in humans, increased proteasome proteolytic activities were found in abdominal muscle from cancer patients [16] and by our group [17] in leg muscle from patients with sepsis and multiple organ failure.

In septic shock with a prolonged critical illness, the depletion of skeletal muscle is of increasing importance for morbidity and mortality. The majority of patients with septic shock treated in the ICU suffer from respiratory dysfunction and need mechanical ventilation. Many of these patients have difficulties in being weaned from the ventilator, thus prolonging ICU treatment and increasing the incidence of pneumonia [18]. The underlying causes of weaning difficulties might be attributed to muscle weakness due to atrophy. Presently, there are no efficient prophylactics or treatment for muscle depletion, although a number of strategies have been suggested over the years. The understanding of the underlying biochemical and molecular mechanisms for muscle protein degradation in catabolic patients is still limited. To be able to better predict and evaluate the outcome of therapeutic efforts for critically ill patients, it is therefore necessary to develop experimental approaches that increase our understanding of how the proteolytic machinery is regulated in the cell.

The aim of the present study was to measure the proteasome proteolytic activity simultaneously in respiratory and leg muscles in patients with septic shock requiring mechanical ventilation in comparison with healthy controls. In previous studies [1–3,17], we observed a large scatter of both protein synthesis rates and proteasome activity in leg skeletal muscle in patients with sepsis compared with control subjects. Thus a second aim of the present study was to elucidate the variation of proteasome activity between duplicate muscle biopsy specimens and between the individual subjects.

MATERIALS AND METHODS

Patients

Two different experiments were performed in the present study, both consisting of one group of patients with septic shock admitted to the general ICU at Karolinska University Hospital, Huddinge, Stockholm, Sweden, and another group of metabolically healthy, age- and sex-matched control patients undergoing elective surgery. In experiment II, the control subjects were recruited at the Ersta Hospital, Stockholm, Sweden. The study protocols conformed to the ethical guidelines of the 1975 Declaration of Helsinki and were approved by the Ethical Committee of Karolinska Institutet, Stockholm, Sweden. All patients or, for the ICU patients, close relatives gave informed consent to participate in the studies after receiving both oral and a standardized written information approved by the Ethical Committee.

Experiment I

Experiment I included ICU patients (n = 10) with septic shock at admittance requiring mechanical ventilation, and control patients (n = 10) undergoing elective surgery. Muscle biopsies were taken from leg muscle and external respiratory muscle. Pre-existing neuromuscular disease, COPD (chronic obstructive pulmonary disease), long-term pre-ICU corticosteroid treatment or severe coagulopathies not enabling muscle biopsies were exclusion criteria. All ICU patients suffered from respiratory dysfunction and multiple organ failure, as indicated by SOFA (sepsis-related organ failure assessment) scores [19] on the day of study (Table 1). All patients had sepsis according to the Bone criteria [20]. All patients were sedated with propofol, together with intermittent doses of analgesics. Mechanical ventilation was pressure support in eight patients and pressure control in two patients. All patients were circulatory stable at the time of study and received low-rate infusion of vasopressor drugs, if any. Three of the patients did not receive any glucocorticoid treatment, four of them received a substitution dose, and three received a therapeutic dose of short duration. Nutrition was given according to the routines of the unit with a target of 20–25 kcal · kg⁻¹ · day⁻¹ (where 1 kcal = 4.184 kJ). Six patients received enteral nutrition, three received combined enteral and parenteral nutrition, and one received parenteral nutrition only. Both enteral and parenteral nutrition contained glutamine. Three of the patients did not survive. Patient #5 died after 1 week, and patients #4 and #8 died 2 months after the study.
Table 1. Characteristics of the ICU patients enrolled in the two experiments

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Diagnosis</th>
<th>Age</th>
<th>Gender</th>
<th>Days in ICU</th>
<th>SOFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment I</td>
<td>Throat abscess/pneumonia</td>
<td>67</td>
<td>Female</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>Liver transplant/quadriplegia</td>
<td>62</td>
<td>Female</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>Bacterial meningitis</td>
<td>51</td>
<td>Female</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>Pneumonia</td>
<td>51</td>
<td>Male</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>Surgical complications</td>
<td>74</td>
<td>Male</td>
<td>22</td>
<td>7</td>
</tr>
<tr>
<td>6</td>
<td>Surgical complications/pneumonia</td>
<td>76</td>
<td>Male</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>Keto acidosis/pneumonia/quadriplegia</td>
<td>40</td>
<td>Male</td>
<td>8</td>
<td>5</td>
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<td>8</td>
<td>Peritoneal abscess</td>
<td>78</td>
<td>Male</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>9</td>
<td>ARDS</td>
<td>80</td>
<td>Male</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>10</td>
<td>Surgical complications/ARDS</td>
<td>67</td>
<td>Male</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Median values</td>
<td></td>
<td>67</td>
<td></td>
<td>8</td>
<td>6</td>
</tr>
</tbody>
</table>

| Experiment II  | Abdominal aortic aneurysm/pneumonia            | 60  | Female | 2           | 7    |
| 2              | Pneumonia                                      | 47  | Male   | 1           | 9    |
| 3              | Stroke/pneumonia/cardiac arrest                 | 63  | Male   | 42          | 8    |
| 4              | Respiratory failure (surgery)                  | 2   | Female | 1           | 5    |
| 5              | Sepsis                                         | 77  | Male   | 2           | 1    |
| 6              | Respiratory failure/COPD                       | 57  | Female | 1           | 6    |
| 7              | Respiratory failure (surgery)                  | 8   | Male   | 2           | 2    |
| 8              | Oesophageal resection/COPD                     | 77  | Male   | 7           | 5    |
| 9              | Respiratory failure/COPD                       | 69  | Female | 1           | 6    |
| 10             | Pneumonia/COPD                                 | 77  | Male   | 3           | 5    |
| 11             | Abdominal aortic aneurysm                      | 73  | Female | 35          | 4    |
| 12             | Multiple rib fractures/respiratory failure     | 74  | Male   | 6           | 9    |
| 13             | Respiratory failure/AMI                        | 66  | Female | 2           | 4    |
| 14             | Pneumonia                                      | 69  | Male   | 2           | 7    |
| 15             | Respiratory failure                            | 48  | Male   | 2           | 7    |
| 16             | Abdominal sepsis (surgery)/COPD                | 71  | Female | 1           | 12   |
| 17             | Abdominal sepsis (surgery)/COPD                | 25  | Female | 6           | 3    |
| Median values  |                                               | 69  |        | 2           | 6    |

The control group consisted of three women and seven men, with a median age of 67 (range 45–87) years, undergoing elective surgery for hernia repair, ilestomy closure, recurrent diverticulitis or colorectal resection. One control patient had a malignant disease without evident spread.

Experiment II

Experiment II included ICU patients with septic shock at admittance \((n = 17)\) and control patients \((n = 10)\) undergoing elective surgery. A muscle biopsy from vastus lateralis was taken from both the left and right leg. Patients with severe liver failure, undergoing dialysis or with impaired coagulation not enabling muscle biopsies, were excluded from the study. None of the patients had long-term pre-ICU systemic corticosteroid treatment. All patients had sepsis according to the Bone criteria [20]. Characteristics of the ICU patients are given in Table 1. According to the SOFA scores, all patients except one had multiple organ failure. All patients were sedated with propofol, together with intermittent doses of analgesics, and required ventilator treatment. At the time of study, all patients were circulatory stabilized, although all but three patients required vasopressor and/or inotropic support. Intravenous short-term corticosteroid treatment was given to four patients, and another six patients were given corticosteroid-substitution therapy. Antibiotics were given to all patients, and nine patients were treated with more than one antibiotic. Antifungal therapy was given to four patients. At the time of study, three of the patients had an indwelling thoracic epidural catheter with continuous infusion of local anaesthetics and short-acting opioids. Total parental nutrition, enteral nutrition or combinations were given to 15 of the patients, and the two remaining patients received 50 mg/ml and 100 mg/ml of glucose respectively. All patients received intravenous insulin to keep blood glucose between

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4 and 8 mmol/l. Four of the patients did not survive (patients 3, 12, 13 and 17) but died 40 days, 3 months, 2 weeks and 1 week respectively, after the study. The control group was entirely separate from that in experiment I and consisted of nine males and one female patient, with a median age of 70 (range 48–76) years. They were all undergoing elective surgery for ventral hernia repair, except for one male patient undergoing laparoscopic cholecystectomy.

**Muscle biopsies**

Leg muscle biopsies were taken percutaneously from the vastus lateralis using a Bergstrom needle. The biopsies were taken using local anaesthesia confined to the skin and fascia only. In the control patients, biopsies were taken just after induction of anaesthesia, but before surgery was started. In experiment I, the muscle covering the rib, mainly the serratus anterior muscle, was identified and used for sampling in order to prevent complications such as pneumothorax and bleeding. The serratus anterior is considered as an accessory muscle for respiration [21]. Biopsies were taken by a surgeon through a 3–5-cm-long incision at the level of the sixth rib in the anterior axillary line. The biopsy was taken using local anaesthesia confined to the skin and subcutaneous tissue without infiltration in the muscle. Local anaesthesia was used in all patients, both the ICU patients with sedation and in the control patients pre-operatively, but after induction of general anaesthesia. A surgical biopsy of 50–200 mg of wet weight was taken. The biopsy site was sutured to prevent bleeding. The incision was finally closed using an intracutaneous technique. No complications were observed with this technique. All biopsies were weighed within 3 min, frozen in liquid nitrogen and then stored at −80 °C until analysis.

Muscle biopsies were homogenized in a buffer [50 mmol/l Tris/HCl (pH 7.2), 1 mmol/l EDTA, 100 mmol/l KCl, 5 mmol/l MgCl2 and 1.8 mmol/l ATP] to obtain a 5 % (w/v) homogenate, and were then centrifuged at 700 g for 10 min to remove cell debris. The resulting supernatant was centrifuged for 10 min at 15 000 g. Glycerol was added to this supernatant (final concentration, 10 % v/v), which was then frozen in liquid nitrogen and stored at −80 °C until analysis. Haemoglobin concentrations in the homogenates were estimated by a haemometer (Zeiss).

**Assay of proteasome activity**

Proteasome chymotrypsin-like activity was measured using the fluorogenic peptide substrate LLVY [succinyl-Leu-Leu-Val-Tyr-AMC (7-amido-4-methylcoumarin); Sigma]. Triplicates of the supernatant obtained after centrifugation at 15 000 g (10 µl; 10–20 µg of protein) were incubated in 50 µl of buffer [50 mmol/l Tris/HCl (pH 7.5), 1 mmol/l ATP, 5 mmol/l MgCl2, 1 mmol/l DTT (dithiothreitol) and 150 µmol/l LLVY]. In parallel incubations, leupeptin or MG115 (Sigma) were added to the incubation system to a final concentration of 100 µmol/l. As a blank, LLVY was incubated in the absence of muscle extract. After incubation at 37 °C for 45 min, the reaction was stopped by adding 1 ml of 100 mmol/l sodium acetate buffer (pH 4.3). Standard curves were prepared using AMC (Sigma). In experiment I, fluorescence was measured with excitation and emission wavelengths of 342 nm and 462 nm respectively, in a PerkinElmer LS 50B spectrofluorimeter, and in experiment II fluorescence was measured with excitation and emission wavelengths of 380 nm and 460 nm respectively, with a FLUOstar OPTIMA (BMG Labtechnologies) spectrofluorimeter. Proteolytic activity was expressed as pmol of AMC released. µg⁻¹ of protein·min⁻¹. In all experiments, chymotryptic activity was measured, as this is the rate-limiting step of protease activities in the proteasome [5], and also the peptide substrate used has been found to be the most proteasome-specific substrate [22].

**Statistical methods**

Student’s t-tests were used to compare the proteasome activity between the ICU patients and their respective control groups. Values are given as means ± S.D.

**RESULTS**

In experiment I, the proteolytic activity of proteasomes was measured in respiratory and leg muscles from patients with sepsis and control patients. In the respiratory muscle, the proteasome activity was 30 % higher in the patients with sepsis compared with control patients (0.24 ± 0.07 compared with 0.18 ± 0.04 pmol·µg⁻¹ of protein·min⁻¹; P = 0.04; Figure 1). The leg muscle showed a similar pattern, with a 45 % higher activity in patients with sepsis compared with controls (0.29 ± 0.10

![Figure 1 Proteasome proteolytic activity in muscle extracts from patients with sepsis (n=10) and controls (n=10)](image)
Proteasome activity in human skeletal muscle

Figure 2 Proteasome proteolytic activity in muscle extracts from patients with sepsis (n = 17) and controls (n = 10)
Muscle biopsies were taken from the left and right leg. Each circle represents an individual subject. Horizontal lines indicate mean values. *P < 0.01 compared with the control value, as determined by a Student's t test.

compared with 0.20 ± 0.05 pmol·µg⁻¹·min⁻¹; P = 0.02; Figure 1).

In experiment II, the variability of the proteasome activity between individuals and between biopsy specimens within an individual was assessed in one group of patients with sepsis and one group of control patients. In accordance with experiment I, the scatter of proteasome activity between individuals was larger in the group of patients with sepsis than in the controls (Figure 2). Biopsies were taken from the left and right leg of each subject to avoid interference from the first biopsy taken and to be able to take muscle biopsies from the same position in the leg. The mean proteasome activity in the patients with sepsis compared with the control group was 0.16 ± 0.06 and 0.10 ± 0.04 pmol·µg⁻¹·min⁻¹ (P = 0.003) respectively, in the left leg, and 0.16 ± 0.06 and 0.11 ± 0.02 pmol·µg⁻¹·min⁻¹ (P = 0.005) respectively, in the right leg (Figure 2). The intra-individual variation in proteasome activity between the two biopsies taken from the legs was fairly high in several of the subjects in both patients with sepsis and controls (Figure 3). Each individual muscle extract was assayed in triplicate for proteasome activity. The mean difference in activity between duplicate biopsies was 44% in the patients with sepsis and 52% in the controls, whereas the mean difference between the highest and lowest activity values in the triplicates was 17% and 24% respectively. This means that the difference in activity between biopsies cannot be explained by the variation in the method. The presence of blood in the muscle extracts differed between the biopsies as judged by haemometer measurements, which might contribute to the variation between the duplicate biopsies, as proteasome activity was expressed per protein. In the patients with sepsis, haemoglobin concentration correlated negatively with proteasome activity ($r = -0.476$, $P < 0.01$; $n = 34$), whereas no such correlation was found in the controls [$r = 0.250$, $P = \text{NS}$ (not significant); $n = 20$].

All determinations of the proteasome proteolytic activity were performed in three parallel incubations: (i) in the presence of the substrate LLVY only, (ii) with the addition of the lysosomal inhibitor leupeptin, or (iii) with the addition of the proteasome inhibitor MG115. When expressed as a percentage of LLVY alone (100%), leupeptin did not influence proteasome activity (103 ± 14%), whereas MG115 almost completely inhibited the activity during incubation (2 ± 3%), a pattern

Figure 3 Proteasome proteolytic activity in muscle extracts from the left and right leg in individual septic patients (1–17) and controls (1–10)
Black bars represent the left leg, and grey bars represent the right leg.
that was the same for all biopsies from both patients with sepsis and controls. This shows that the proteolytic activity measured in the assay represents proteasome activity only.

**DISCUSSION**

Catabolic conditions, including sepsis, trauma and cancer, are associated with loss of skeletal muscle tissue [23]. In the present study, two groups of ICU patients with sepsis and multiple organ failure were found to have increased proteasome proteolytic activity in both leg and respiratory muscles. Compared with the control groups, the mean proteasome activity of ICU patients was 30 % higher in the respiratory muscle and 45–55 % higher in the leg muscle.

From the results in the present study, it is not possible to ascertain whether the increased activity is a result of an increased number of proteasomes in the muscle cell or of an increased intrinsic proteolytic activity of the proteasomes. In a recent study of cancer patients with weight loss [13], concomitant increases in mRNA levels and protein levels for the proteasome subunits C2 and C5 were found. However, other studies have failed to show a correlation between mRNA expression and translation products. After mitogen-induced proliferation of human T-lymphocytes, increased levels of mRNA for proteasome subunits C2, C3 and C5 were detected, but no increase in the cellular content of proteasomes was seen [24]. Also, in a rat model of sepsis, no correlation between mRNA expression and proteasome content in skeletal muscle was observed [7]. In mammalian cells, the 20 S proteasomes exist in several different forms which exhibit different proteolytic activities. Six different forms of proteasome subtypes have been separated from rat skeletal muscle [25]. After induction of diabetes mellitus, a redistribution of proteasome subtypes was found in rat muscle [26]. Thus the increase of proteasome activity seen in the ICU patients could be an effect of an increased number of proteasomes in muscle cells, a redistribution of proteasome subtypes exhibiting a higher intrinsic proteolytic activity or maybe a combination of both.

Patients with sepsis and multiple organ failure usually suffer from respiratory impairment and, therefore, need mechanical ventilation. As many as 20 % of these patients experience difficulties in weaning off the ventilator [18]. Atrophy of respiratory muscles is thought to be one of the underlying causes of the development of respiratory difficulties. In a group of patients with mild-to-moderate COPD, a reduction in diaphragm myosin heavy chain content, together with an increase in ubiquitin-protein conjugation in diaphragm muscle homogenates, indicated an enhanced protein degradation [27]. On the other hand, mechanical ventilation alone seems to induce respiratory muscle atrophy and, thus, may contribute to prolonged ventilation treatment. Studies of the diaphragm from ventilated rats show a decrease in myofibrillar protein and an increase in proteasome activity [28,29]. In the present study, we show that patients with sepsis-induced multiple organ failure and needing mechanical ventilation, in addition to general muscle wasting, also had an increased proteasome activity in respiratory muscles. However, the muscle used in the present study, serratus anterior, is an accessory respiratory muscle and results might be different in the main respiratory muscles, i.e. diaphragm and intercostal muscles.

In experiment II, the inter- and intra-individual variation of proteasome activity was investigated in a group of patients with sepsis and a control group. The greater variation in proteasome activity between patients with sepsis compared with controls may be dependent on the size of muscle mass at the onset of disease. One theory is that muscle wasting might be beneficial during sepsis, supplying splanchnic organs and immune cells with increased amounts of amino acids released from skeletal muscle. If muscle mass is small, a proportionally larger amount of muscle might have to be degraded in order to provide sufficient amounts of amino acids for other organs. It is known that lean body mass decreases with age, but no correlation was found between the age of the ICU patients and proteasome activity (r = -0.045, P = NS). Nor was there any correlation between proteasome activity and SOFA scores on the study day (r = -0.286, P = NS). When considering the length of stay in the ICU, a correlation was found (r = 0.621, P < 0.01), but two patients that stayed for 42 and 35 days respectively, were the determining factor. However, this correlation was not found in the remaining 15 patients that had stayed between 1–7 days in the ICU (r = 0.076, P = NS). Some of the patients received short-term glucocorticoid treatment at different doses, and glucocorticoids are known mediators of muscle proteolysis [9]; however, we found no correlation between proteasome activity and glucocorticoid dose when comparing patients with no treatment, low-dose substitution therapy and high-dose treatment (r = -0.132, P = NS). Of course, ICU patients with sepsis have a great variety of underlying diseases that probably contributed to the large variation.

Fractional protein synthesis of total protein in leg muscle varies by 6 % between duplicate biopsy specimens [30]. The variation of proteasome activity between the two biopsy specimens in experiment II was significantly larger than this. It probably cannot be explained by skewed distribution of fibre type between biopsies, as the human leg muscle consists of a mixture of slow- and fast-fibre types that are evenly distributed within vastus lateralis [31,32]. The presence of blood in the biopsies can, to some extent, explain the results, at least in the patients with sepsis, where high haemoglobin concentrations in the homogenates correlated negatively with proteasome activity. However, no such correlation was found...
in the controls. In both experiments in the present study, we did not have access to large enough biopsy specimens and, therefore, we decided to measure the proteasome activity in crude homogenate fractions (15 000-g supernatants). Probably, the variation between duplicate biopsies would decrease if activity was measured in fractions of proteasomes isolated by sequential ultracentrifugation, as performed in a previous study [17], but this method requires muscle biopsies of at least 70 mg. Nevertheless, in spite of the large variation between duplicate biopsies in both groups of study subjects in the present study, it was still possible to detect a statistically significant difference in proteasome activity between the patients with sepsis and their controls. Thus proteasome activity measurements in crude extracts still could be an option in studies with limited size of biopsy specimens.

In conclusion, our results have shown that proteasome activity was significantly increased in skeletal muscle during sepsis and multiple organ failure, and we have shown for the first time that both respiratory and leg muscles were affected. The increased proteasome activity in respiratory muscle indicates an accelerated protein degradation that may contribute to respiratory dysfunction in patients that are treated in the ICU. Since the protein synthesis rate in skeletal muscle of these patients was not changed, increased proteasome activity supports the hypothesis that muscle wasting during severe catabolic conditions, such as sepsis and multiple organ failure, is the result of increased protein degradation.

ACKNOWLEDGMENTS

We thank Viveka Gustavsson at Karolinska University Hospital Huddinge, and Ann-Sofie Andersson and Nina Johansson at Ersta Hospital for excellent nursing assistance.

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Received 19 September 2006/22 November 2006; accepted 22 November 2006
Published as Immediate Publication 22 November 2006, doi:10.1042/CS20060265