SHORT COMMUNICATION

Myofibrillar protein catabolic rates in cirrhotic patients with and without muscle wasting

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Summary

1. The urinary excretion of 3-methylhistidine and creatinine was measured in 15 controls and in two groups of 15 patients with liver cirrhosis, with and without severe muscle wasting. All subjects were on a meat-free diet. The values obtained were used to calculate the fractional catabolic rate of myofibrillar protein.

2. In patients without muscle wasting 3-methylhistidine excretion was high in the presence of normal urinary creatinine. The fractional breakdown rate was significantly increased as compared with that of controls.

3. In patients with severe muscle wasting 3-methylhistidine excretion was normal and urinary creatinine was remarkably reduced. The myofibrillar catabolic rate was further increased compared with that of controls and of the other group of patients.

4. 3-Methylhistidine and creatinine excretion allow a complete evaluation of myofibrillar protein degradation, which appears to be remarkably increased in cirrhotic patients. The relevance of increased myofibrillar protein turnover in muscle wasting of subjects with advanced cirrhosis remains to be determined.

Key words: cirrhosis, creatinine, 3-methylhistidine, muscle protein.

Introduction

The urinary excretion of 3-methylhistidine is increasingly being used to indicate the rate of muscle protein breakdown [1–4]. In a previous paper a significant increase in 3-methylhistidine elimination was demonstrated in patients with cirrhosis [5], possibly as a result of alterations in pancreatic glucoregulatory hormones [6]. These data are consistent with the clinical observation of a progressive reduction of the lean body mass, which becomes evident in advanced cirrhosis. In patients with severe muscle wasting 3-methylhistidine excretion may fall to normal levels because of lack of substrates. Only a complete assessment of the myofibrillar protein catabolic rate may clearly indicate the extent of muscle catabolism.

The aim of this study was to calculate the myofibrillar protein catabolic rate in patients with liver cirrhosis with and without clinical evidence of severe muscle wasting.

Methods

Fifteen subjects (eight males and seven females, aged 38–64 years (median 56), mean height 1.68 m (SD 0.08), mean weight 67.3 kg (SD 7.4)), with normal liver and kidney function, served as controls. Nine of them had been admitted to the hospital for functional disorders of the gastrointestinal tract, and were taking no drugs; six had mild cardiovascular disorders and were taking diuretics.

Two groups of cirrhotic patients, divided on the basis of their body weight, were also examined. The first group consisted of eight males and seven females, median age 53 years (range 32–74), whose weight varied within 10% of the ideal body weight [mean height 1.69 m (SD 0.07); mean weight 69.0 kg (SD 8.8)]. They never showed...
ascites. Pertinent laboratory data in these patients were as follows (mean ± SEM): albumin, 3.5 ± 0.2 g/dl; aspartate and alanine transaminases, 39 ± 10 and 27 ± 7 units/l respectively (normal values <12 units/l); alkaline phosphatase, 45 ± 8 units/l (normal values <28 units/l); prothrombin activity, 63 ± 4%. 

The second group included 15 cirrhotic patients (eight males and seven females, aged 37–72 years, median 52) with a remarkable reduction of their lean body mass (body weight <80% of ideal body weight). Their mean height was 1.68 m (SD 0.07) and mean weight was 54.4 kg (SD 6.9). They periodically had ascites, which was clinically undetectable at the time of the study. Most of them were under diuretic treatment (spironolactone up to 300 mg/day and/or frusemide 25–50 mg/day). Results of liver-function tests in this group were: albumin, 2.6 ± 0.2 g/dl; aspartate and alanine transaminases, 31 ± 8 and 22 ± 10 units/l respectively; alkaline phosphatase, 59 ± 14 units/l; prothrombin activity, 53 ± 4%.

In the two groups, liver cirrhosis was proved by liver biopsy taken, in most cases, under laparoscopic control. All patients had normal renal function (serum creatinine levels <0.11 mmol/l). None received corticosteroid treatment. No changes in therapy were performed throughout the study.

Both controls and patients were hospitalized and consumed a meat-free diet containing 110–130 kJ and 1 g of protein/kg body weight for the 2 days before the analyses as well as for the 3 consecutive days of urine collection.

Consent for participation in the study was obtained from all subjects, and the nature and purpose of the study were explained to get their active cooperation in maintenance of dietary regimen and careful attention in urine collection.

Twenty-four hour urine collection started from 08.00 hours, after 2 days of the standard diet, and was carried on for 3 consecutive days. HCl was used as a preservative. Analysis of 24 h 3-methylhistidine was performed by means of a 3A 28 Carlo Erba amino acid analyser (Carlo Erba Strumentazione, Rodano, Italy) [5]. Urinary creatinine was determined as well [7].

The myofibrillar catabolic rate was calculated by assuming that 1 g of urinary creatinine/day is equivalent to 20 kg of muscle [8], that proteins constitute 20% of muscle mass (65% myofibrillar protein), and that the mean concentration of 3-methylhistidine in human muscle is 4.2 μmol/g of protein [3].

Results are expressed as means ± SEM of the mean values from individual subjects, calculated from data obtained over 3 consecutive days. Differences between mean values were tested for significance by means of Student’s t-test for unpaired data.

**Results**

The results are reported in Table 1.

Urinary creatinine, muscle mass and myofibrillar protein mass appeared to be similar in controls and in cirrhotic patients with normal body weight. In patients with reduced lean body mass they were lower, confirming the clinical observation of muscle wasting and decreased body weight.

As for the urinary excretion of 3-methyl-

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**Table 1. Urinary excretion of 3-methylhistidine and creatinine, and myofibrillar protein catabolic rate in controls and in the two groups of cirrhotic patients with normal body weight or with severe muscle wasting**

Results are expressed as means ± SEM of the mean value of individual subjects (calculated from data obtained over 3 consecutive days). Day-to-day variations were within 15%. Significances of differences, as compared with control values, are given in parentheses; differences compared with the corresponding value of cirrhotic patients with normal body weight:

*P < 0.05; **P < 0.005.

<table>
<thead>
<tr>
<th>(1) Creatinine excretion (mmol/day)</th>
<th>(2) Muscle mass (kg)</th>
<th>(3) Myofibrillar protein mass (g/day)</th>
<th>(4) 3-Methylhistidine excretion (μmol/g)</th>
<th>(5) Myofibrillar protein catabolism (g/day)</th>
<th>(6) Myofibrillar catabolic rate (%/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Controls (n = 15)</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>11.9 ± 0.6</td>
<td>27 ± 2</td>
<td>3.5 ± 0.2</td>
<td>184 ± 20</td>
<td>28 ± 3</td>
<td>0.81 ± 0.08</td>
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<tr>
<td><strong>Cirrhotic patients</strong></td>
<td></td>
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<tr>
<td>All patients (n = 30)</td>
<td>10.0 ± 0.6</td>
<td>23 ± 2</td>
<td>3.0 ± 0.2</td>
<td>253 ± 17</td>
<td>39 ± 3</td>
</tr>
<tr>
<td>Normal body weight (n = 15)</td>
<td>12.5 ± 0.5</td>
<td>28 ± 1</td>
<td>3.7 ± 0.2</td>
<td>288 ± 25</td>
<td>44 ± 4</td>
</tr>
<tr>
<td>Severe muscle wasting (n = 15)</td>
<td>7.4 ± 0.5**</td>
<td>17 ± 1**</td>
<td>2.2 ± 0.2**</td>
<td>218 ± 19*</td>
<td>34 ± 3*</td>
</tr>
</tbody>
</table>

(P < 0.001) (P < 0.001) (P < 0.001) (P < 0.001) (P < 0.001) (P < 0.001)
histidine and myofibrillar protein catabolism, both were significantly raised in cirrhotic patients with normal body weight in comparison with values in controls, and fell to nearly normal values in severely cachectic patients.

The myofibrillar protein catabolic rate was found to be high in all groups of patients with cirrhosis, and mainly in patients with severe muscle wasting, who differed significantly from both control subjects ($P < 0.001$) and from the other group of patients ($P < 0.05$).

### Discussion

3-Methylhistidine is formed by the methylation of peptide-bound histidine of actin and myosin [9, 10]. After myofibrillar catabolism, 3-methylhistidine cannot be reutilized, and is excreted in the urine in the form of a free amino acid [11]. Besides skeletal muscle, a few sources of endogenous 3-methylhistidine have been identified, including the skin and gastrointestinal muscle [12]. The 3-methylhistidine content of these tissues is low, but their contribution to total 3-methylhistidine excretion was estimated to be as much as 16%, because of their more rapid turnover. These and other data [13] raised doubts about the validity of 3-methylhistidine measurement for the assessment of muscle protein turnover. Current evidence indicates that skeletal muscle breakdown accounts for about 75% of total urinary 3-methylhistidine [14, 15], which may be assumed as a reliable index of muscle protein catabolism. Only diet proved to affect 3-methylhistidine excretion considerably [16], and therefore estimates of muscle catabolism with this technique require that subjects are fed with a meat-free diet, or that the amount of meat is strictly quantified [17].

The amount of urinary 3-methylhistidine varies according to myofibrillar protein mass and in subjects with severe muscle wasting low excretion values may be present in spite of a high catabolic rate [18]. In these subjects the ratio of 3-methylhistidine to creatinine may give a better estimate of myofibrillar protein turnover, but simple calculations may also allow one to obtain the apparent myofibrillar protein catabolic rate. In far-advanced cirrhotic patients with a severe reduction of the lean body mass, 3-methylhistidine excretion might fall to normal. In the present study we tried to assess the turnover of myofibrillar proteins in cirrhotic patients with normal body weight or with muscle wasting. In the first group we confirmed previous data [6] and demonstrated an increased myofibrillar protein turnover. Control values are in the same range as reported by other authors for subjects on a meat-free diet [4, 19]. Small differences in the catabolic rate, as compared with the figure from other groups [20], are due to the different figure which they used for 3-methylhistidine content in mixed muscle. In far-advanced patients urinary creatinine was severely reduced, in agreement with the clinical observation of muscle wasting. 3-Methylhistidine was reduced as well; however, these patients proved to have myofibrillar protein catabolic rates significantly higher than those of controls or of compensated cirrhotic patients.

Calculations of myofibrillar protein catabolic rate, by means of urinary creatinine and 3-methylhistidine excretion, are based on several assumptions throughly reviewed by McKeran et al [4]. We further assumed that 3-methylhistidine content of muscle protein in cirrhotic patients is the same as in controls, but no biochemical studies have so far been reported on this topic.

A further assumption is that urinary creatinine strictly correlates with muscle mass in patients with cirrhosis. Fat-free weight, as assessed by densitometry [18] or by $^{40}$K radioactivity [21], strictly correlates with 24 h urinary creatinine in normals, but no data were reported for cirrhotic patients. The values we obtained in our patients with normal renal function, both with and without muscle wasting, are in agreement with this assumption.

The finding of increased myofibrillar protein catabolic rate in patients with advanced cirrhosis and muscle wasting is surprising. In normal subjects undergoing starvation 3-methylhistidine excretion progressively reduces, as a result of decreased muscle protein catabolism [1]. This possibly represents an adaptive mechanism to spare proteins and essential amino acids. The mechanism and the causes by which cirrhotic patients fail to spare myofibrillar protein in the presence of reduced lean body mass is unknown, and possibly derives from complex metabolic disturbances. Further studies are needed to evaluate the rate of muscle synthesis in the presence of increased muscle catabolism. Although protein synthesis seems to play a major role in regulating muscle mass [22], in patients with cirrhosis increased myofibrillar
catabolic rate may contribute to the clinical picture of wasting.

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References


