Comparison of human myofibrillar protein catabolic rate derived from 3-methylhistidine excretion with synthetic rate from muscle biopsies during L-[α-15N]lysine infusion

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Summary

1. Urine was collected in five healthy men over 10–14 days, with fasting blood samples on days 1, 5 and 10, whilst they consumed a standard creatine-free diet, which was quantitatively related to their body surface area.

2. The urinary excretion of 3-methylhistidine fell to a plateau by day 5 in all subjects. Myofibrillar protein catabolic rate calculated from the mean value of 3-methylhistidine excretion from day 5 to day 10 averaged 1.21 g day⁻¹ kg⁻¹ body weight. The average turnover of muscle myofibrillar protein was calculated to be 2.16%/day.

3. From a previous study using continuous intravenous infusion of L-[α-15N]lysine with serial muscle biopsies on the same subjects, the mean myofibrillar protein synthetic rate was calculated to be 0.82 g day⁻¹ kg⁻¹ body weight, and the mean turnover rate was 1.47%/day of total muscle myofibrillar protein.

4. The estimations of myofibrillar protein turnover rate derived from the two methods are compared and the differences discussed.

Key words: L-[α-15N]lysine, 3-methylhistidine, myofibrillar protein.

Introduction

Muscle proteins, which constitute 40–45% of the total protein mass of the body, are a heterogeneous collection of individual proteins, each with specific control mechanisms regulating synthesis and possibly degradation rates. The catabolic rates of muscle myofibrillar protein in man have been derived in vivo from measurements of 3-methylhistidine excretion in urine by Young, Munro & Scrimshaw (1975). This amino acid is present in the actin of all muscles and the myosin of white muscle fibres, with most being in skeletal muscle (Haverberg, Omstedt, Munro & Young, 1975). It is quantitatively excreted in the urine, is neither reutilized for protein synthesis (Young, Alexis, Baliga, Munro & Muecke, 1972) nor oxidatively metabolized (Long, Haverberg, Kinney, Young, Munro & Geiger, 1975). Its excretion has been taken as an index of muscle myofibrillar protein breakdown. The method may be limited by inability to measure fractional catabolic rates of sarcoplasmic and stromal muscle proteins. We have previously described the direct measurement of synthetic rates of sarcoplasmic and myofibrillar muscle proteins in vivo in man, with estimation of their individual turnover rates and percentage contribution to whole-body protein synthesis (Halliday & Mckeran, 1975). We now compare measurements of muscle myofibrillar protein synthetic rate in man from serial muscle biopsies during continuous intravenous infusion of L-[α-15N]lysine with those derived for myofibrillar protein.
catabolic rate from urinary 3-methylhistidine excretion in the same subjects.

**Patients and methods**

Five healthy male volunteer subjects were re-studied (Halliday & McKeran, 1975). The purpose of the investigation was explained to each individual and their full consent was obtained. A standardized creatine-free diet was given for 10 days. Complete 24 h urine collections were made for 10 days, and in subject no. 5, urine collection was continued for a further 3 days on returning to a normal diet. The creatine-free diet contained 40 g of protein/m² and 5 MJ/m² body surface area. Fasting blood samples were taken on days 1, 5 and 10 of the diet.

Urinary 3-methylhistidine was measured by a Technicon TSM amino acid analyser. The standard cycle for the analysis of physiological fluids was curtailed so that only the basic amino acids were quantified. L-Canavanine was used as an internal standard. The reproducibility of the method was within 5% as determined by replicate analyses of standard mixtures.

A portion of urine from each timed collection, containing approximately 1 mg of nitrogen, was digested and measured by Kjeldahl analysis with selenium dioxide as catalyst. Urinary creatinine was measured by the method of Edwards & Whyte (1958).

Plasma amino acid chromatograms were run on fasting blood samples taken on days 1, 5 and 10 of the study. Plasma proteins were precipitated by adding an equal volume of aqueous 20% (w/v) salicylsulphonic acid solution to the plasma. The resulting mixture was centrifuged for 10 min at 2000 rev./min at 4°C and the supernatant analysed by the programme for physiological fluids on the Technicon TSM analyser. Norleucine was used as internal standard.

Samples of plasma obtained on days 1, 5 and 10 of the creatine-free diet were analysed on a Vickers M-300 multichannel analyser for urea, uric acid and proteins.

Myofibrillar protein catabolic rate (expressed as g of myofibrillar protein day⁻¹ kg⁻¹ body weight) was calculated from: 3-methylhistidine excretion (μmol/24 h)/(2·71 × weight (kg)). This assumes that the concentration of 3-methylhistidine in mixed proteins of human muscles was 1·76 μmol/g of protein (Asatoor & Armstrong, 1967), and myofibrillar protein constituted 65% of total muscle protein.

The percentage turnover per day of muscle myofibrillar protein was calculated from:

\[
\text{myofibrillar protein catabolic rate (g/day)} \times 100
\]

\[
\frac{\text{total muscle myofibrillar protein}}{\text{g/d}}
\]

This assumes that 8·84 mmol of creatinine excreted was equivalent to 20·0 kg of muscle (Graystone, 1968) and protein constituted 20% of muscle mass, of which 65% was myofibrillar protein.

**Results**

On a creatine-free diet, 3-methylhistidine excretion in urine fell, reaching a plateau by day 5 (Fig. 1). The dietary contribution to 3-methylhistidine excretion was variable and was particularly marked in subject no. 5 (Fig. 1). The mean value of 3-methylhistidine excretion from day 5 to day 10 of collection (Table 1) was used to calculate the myofibrillar protein catabolic rate, which was on average 1·21 g day⁻¹ kg⁻¹ body weight (range 0·97–1·54; Table 2). The turnover of muscle myofibrillar protein was calculated to be 2·16%/day (range 1·87–2·47; Table 2).

In our previous study (Halliday & McKeran, 1975) we estimated the fractional synthetic rate for muscle myofibrillar protein, which we have used to calculate the turnover of muscle myofibrillar protein by using the estimates of muscle mass and total muscle myofibrillar protein derived from the 10 days of urine creatinine measurement in the present study. This was necessary because of the known variability of 24 h urine creatinine excretion in normal subjects (Greenblatt, Ransil, Harnatz, Smith, Duhme & Koch-Weser, 1976), and the 10 days of creatinine estimations in the present study probably gave a more accurate estimate of muscle mass than those derived from the previous study, which were based on fewer measurements. The synthetic rate of muscle myofibrillar protein derived from the fractional synthetic rate for muscle myofibrillar protein of the first study was calculated to be 0·82 g day⁻¹ kg⁻¹ body weight (range 0·60–1·39; Table 2), and the turnover rate as 1·47%/day (range 1·10–2·44; Table 1) of total muscle myofibrillar protein.

There was no significant change in the urinary nitrogen excretion in the five subjects throughout the creatine-free diet. There was also no significant change in the plasma urea, uric acid, plasma proteins or amino acids. Plasma 3-methylhistidine concentrations were less than 5 μmol/l throughout.
Myofibrillar protein catabolism

FIG. 1. Excretion of 3-methylhistidine on a creatine-free diet in the five subjects studied (○, no. 1; ▲, no. 2; ■, no. 3; ○, no. 4; Δ, no. 5). Details of the subjects are given in Table 1.

TABLE 1. Clinical data for the five men studied, with their mean creatinine and 3-methylhistidine excretion on a creatine-free diet

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>Body surface area (m²)</th>
<th>Creatinine excretion (g/day)</th>
<th>Muscle mass (kg)</th>
<th>Total muscle myofibrillar protein (kg)</th>
<th>3-Methylhistidine excretion (µmol/day)</th>
<th>Molar ratio (3-methylhistidine/creatine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>37</td>
<td>159</td>
<td>62.5</td>
<td>1.52</td>
<td>1.37</td>
<td>27.4</td>
<td>3.56</td>
<td>193.83</td>
<td>0.016</td>
</tr>
<tr>
<td>2</td>
<td>33</td>
<td>175</td>
<td>80.4</td>
<td>1.95</td>
<td>1.69</td>
<td>33.8</td>
<td>4.39</td>
<td>258</td>
<td>0.017</td>
</tr>
<tr>
<td>3</td>
<td>33</td>
<td>171</td>
<td>63.0</td>
<td>1.74</td>
<td>1.29</td>
<td>25.8</td>
<td>3.35</td>
<td>205.2</td>
<td>0.018</td>
</tr>
<tr>
<td>4</td>
<td>48</td>
<td>183</td>
<td>74.0</td>
<td>1.94</td>
<td>1.48</td>
<td>29.6</td>
<td>3.85</td>
<td>195.18</td>
<td>0.015</td>
</tr>
<tr>
<td>5</td>
<td>34</td>
<td>195</td>
<td>64.4</td>
<td>1.96</td>
<td>1.54</td>
<td>30.8</td>
<td>4.00</td>
<td>268.17</td>
<td>0.020</td>
</tr>
</tbody>
</table>

Mean 0.017

TABLE 2. Comparison of the calculated breakdown rate of muscle myofibrillar protein derived from 3-methylhistidine excretion with that obtained during continuous intravenous infusion of [15N]lysine for the five men listed in Table 1

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>3-Methylhistidine excretion</th>
<th>[15N]Lysine infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myofibrillar protein catabolism (g/day)</td>
<td>Turnover of myofibrillar protein (%/day)</td>
<td>Myofibrillar protein catabolic rate (g day⁻¹ kg⁻¹ body wt.)</td>
</tr>
<tr>
<td>-------------</td>
<td>-----------------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>1</td>
<td>71.52</td>
<td>2.01</td>
</tr>
<tr>
<td>2</td>
<td>95.20</td>
<td>2.17</td>
</tr>
<tr>
<td>3</td>
<td>75.72</td>
<td>2.26</td>
</tr>
<tr>
<td>4</td>
<td>72.02</td>
<td>1.87</td>
</tr>
<tr>
<td>5</td>
<td>98.96</td>
<td>2.47</td>
</tr>
</tbody>
</table>

Mean 2.16 1.21 1.47 0.82
Discussion

The amino acid 3-methylhistidine is a characteristic component of actin and myosin in both skeletal and cardiac muscle. It has thus been used as a marker of myofibrillar protein catabolism in a variety of clinical conditions. Excretion is reduced in protein-calorie malnutrition in children and increases significantly after treatment (Rao & Nagabhushan, 1973), falls progressively during starvation (Young, Haverberg, Bilmazes & Munro, 1973) and is increased after normoketonaemic injury in man (Williamson, Farrell, Kerr & Smith, 1977) and in Duchenne muscular dystrophy (relative to the reduced muscle mass: McKeran, Halliday & Purkiss, 1977). A high-meat-protein diet increases the amount excreted (Block, Hubbard & Steele, 1965) and the fall in 3-methylhistidine excretion during the first 4 days on a creatine-free diet in four subjects in this present study was taken to represent the dietary component of 3-methylhistidine, as there was no change in creatinine or nitrogen excretion. Excluding subject no. 5, who showed the greatest fall to a plateau value, for which no incidental metabolic or muscular explanation could be found, the dietary contribution to 3-methylhistidine excretion on a normal diet was small.

In estimating myofibrillar protein catabolism from 3-methylhistidine excretion it is assumed that the concentration of 3-methylhistidine in mixed muscle proteins is reasonably constant, and that all striated muscle myofibrillar protein is catabolized at a similar rate. Studies in animals (Buttery, Beckerton, Mitchell, Davies & Annison, 1975) indicate that the fractional protein synthetic rates for different skeletal muscles are approximately equal, although cardiac muscle has approximately twice the rate of voluntary striated muscle. Estimates of the turnover of muscle myofibrillar protein from 3-methylhistidine excretion are based upon muscle-mass estimations derived from creatinine excretion. To compare the rate of myofibrillar protein turnover derived from the two methods, we used the mean value of creatinine excretion over the 10 days because of the known variability of 24 h urine creatinine excretion in normal subjects (Greenblat, Ransil, Harmatz, Smith, Duhme & Koch-Weser, 1976). Lean body mass as determined by 40K radioactivity counting is highly correlated with urinary creatinine excretion \( r = 0.988 \) and technical errors can be reduced by using three consecutive collections of urine (Forbes & Bruining, 1976).

We have compared two different methods of estimating myofibrillar protein turnover in the same subjects: one based on catabolism, and the other on the synthetic rate of myofibrillar protein. These two measurements were, however, separated in time by approximately 2 years. A value of 2-16%/day (range 1-87-2-47) was found for the turnover of muscle myofibrillar protein derived from 3-methylhistidine excretion, compared with 1-47%/day (range 1-10-2-44) from continuous infusion of L-$\alpha$-[15N]lysine with serial muscle biopsies. There was no rank correlation when individual subjects' results were compared but, with the exception of subject no. 1, who had received an initial pulse label of [15N]lysine (Halliday & McKeran, 1975), the estimates derived from 3-methylhistidine excretion were higher than those derived from continuous intravenous lysine infusion (mean 82%, range 45–124%, \( n = 4 \)).

Not all the 3-methylhistidine excreted in the urine is derived from breakdown of muscle protein. 3-Methylhistidine has been found in histones, in the actin and myosin-like brain proteins, human platelets and fibroblasts (Young, Haverberg, Bilmazes & Munro, 1973). These extramuscular sources of 3-methylhistidine may account for a small part of the difference in myofibrillar protein turnover rates. Animal evidence is accumulating which suggests that muscle protein mass is regulated primarily through alterations in the rate of protein synthesis (Milward, Garlick, Nnanyelugo & Waterlow, 1976). It is therefore possible that measurements of protein synthetic rate in man are more important in monitoring short-term changes in the body's response to environmental effects, whereas 3-methylhistidine excretion gives a better indication of the longer-term adjustments of muscle protein turnover. Our two studies were separated by 2 years and performed under different conditions. It is possible that the experimental conditions during the first investigation temporarily depressed muscle protein synthesis, but there remains the possibility that 3-methylhistidine excretion may not be a true indicator of muscle protein breakdown. However, the estimates for muscle myofibrillar protein turnover derived from the two methods are of the same order of magnitude, giving 46 days for whole-body myofibrillar protein turnover as derived from 3-methylhistidine, and 68 days for that from continuous infusion of [15N]lysine. Final validation of the value of 3-methylhistidine excretion as a marker of muscle myofibrillar protein catabolism will require comparisons of muscle protein synthetic rates with directly measured catabolic rates.
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in animals, under different environmental conditions in health and disease.

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References


