Increased myofibrillar protein catabolism in Duchenne muscular dystrophy measured by 3-methylhistidine excretion in the urine

R. O. McKERAN, D. HALLDAY, AND P. PURKISS

From the Clinical Research Centre, Harrow, and University College Hospital, London

Summary Myofibrillar protein catabolic rate was calculated in seven patients with Duchenne muscular dystrophy from the amount of 3-methylhistidine excreted in the urine, and found to be over three times that found in a control series when expressed as the percentage of myofibrillar protein catabolised per day. It is suggested that measurement of myofibrillar protein catabolic rate may add a useful parameter in the study of muscle disorders.

Duchenne muscular dystrophy is characterised pathologically by progressive muscle cell death with leakage of soluble enzymes into the plasma. Elevated levels of serum creatine phosphokinase (CPK, adenosine 5' triphosphate-creatine phosphotransferase, EC 2.7.3.2) have been of great value in the diagnosis of the affected hemizygote, and in carrier detection. Recent observations (Ionasescu, 1975) have demonstrated abnormalities of protein synthesis in Duchenne muscular dystrophy, with high rates of synthesis of collagen, and low rates of non-collagen protein synthesis in the polyribosomes isolated from patients' muscles. A similar increased polyribosomal protein synthesis was found in the carriers for the X-linked gene (Ionasescu et al., 1971; Ionasescu et al., 1973).

This study was undertaken to see whether calculation of myofibrillar protein catabolic rate from 3-methylhistidine excretion in the urine could be used as a simple method of detecting abnormalities in protein catabolism of muscle in the affected hemizygote, and thus of potential value in the diagnosis of the heterozygote, and possibly the affected hemizygote in utero.

Patients and methods

Seven institutionalised patients with classical sex-linked Duchenne muscular dystrophy were studied. None of the patients were ambulant at the time of study and they were on a normal ward diet with no additional vitamin or drug therapy.

Urinary 3-methylhistidine was measured by means of a Technicon TSM amino acid analyser. The standard physiological fluid analysis cycle was curtailed so that only the basic amino acids were quantitated with L-canavanine as an internal standard. Urinary creatinine was measured by the method of Edwards and Whyte (1958).

Myofibrillar protein catabolic rate was calculated from the following formula, assuming the concentration of 3-methylhistidine in mixed proteins of human muscles is 1.76 μmole/g protein (Asatoor and Armstrong, 1967), and that myofibrillar protein constitutes 65% of total muscle protein.

\[
\text{3-MeHist excretion (μmol/24h)} \times \frac{1}{2.71} \times \frac{\text{Weight (kg)}}{\text{g of myofibrillar protein/kg body wt/day}}
\]

The percentage turnover per day of myofibrillar protein was calculated from the following formula, assuming 1 g of creatinine excreted is equivalent to 20 kg of muscle, creatinine excretion is a measure of lean body mass in muscle diseases (Ryan et al., 1957), and protein constitutes 20% of muscle mass of which 65% is myofibrillar protein.

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

Our results on these seven patients together with age- and sex-matched controls are summarised in
the Table. Myofibrillar protein catabolic rate was found to be 0.72 g/kg body wt/day (range 0.27–1.26). Muscle mass was determined from creatinine excretion over 24 hours assuming 1.0 g of creatinine equal to 20 kg of muscle (Graystone, 1968) and myofibrillar protein to constitute 65% of total muscle protein. Myofibrillar protein catabolic rate was calculated as 7.3%/day of total muscle myofibrillar protein (range 2.92–14.05) which was over three times the normal value in healthy male volunteers (Young et al., 1975; McKeran et al., 1977). When allowance was made for the possibility of incomplete 24h urine collection, by expressing the relationship of 3-methylhistidine to creatinine as their mole ratio, this similarly was found to be over three times the control value, confirming the threefold increase in myofibrillar protein catabolism in Duchenne muscular dystrophy.

Discussion

Recent estimates of myofibrillar protein catabolic rates in man have used measurements of 3-methylhistidine excretion in urine (Young et al., 1973; McKeran et al., 1977), since this amino acid is present in the actin of all muscles and the myosin of white muscle fibres, with a major proportion in skeletal muscle (Haverberg et al., 1975), it is quantitatively excreted in urine and is not reused for the purposes of protein synthesis (Young et al., 1972), or metabolised via oxidative pathways (Long et al., 1975). In a recent series of studies (Halliday and McKeran, 1975; McKeran et al., 1977), the turnover of muscle protein, and the percentage contribution to whole body protein synthesis from muscle protein synthesis measured directly from serial muscle biopsies during continuous infusion of L-(α-15N) lysine, was compared with myofibrillar protein catabolism calculated from 3-methylhistidine excretion.

Previous reports of 3-methylhistidine excretion in Duchenne muscular dystrophy have shown increased amounts, but it has not been appreciated that 3-methylhistidine can be used as a marker of myofibrillar protein catabolic rate (Bank et al., 1971, Pennington, 1974). When myofibrillar protein catabolic rate was calculated as g/kg of body weight/day in the present study, the results fell either within or below the normal range (Table). When expressed as the percentage turnover of total muscle myofibrillar protein, thus taking account of the reduced muscle mass, the true situation of increased muscle myofibrillar protein catabolism became apparent. The finding of decreased non-collagen protein synthetic rate in vitro (Ionesescu, 1975) coupled with our observations of increased myofibrillar protein catabolism are compatible and point to progressive muscle cell death with leakage of soluble enzymes into the plasma. A more fundamental abnormality of the assembly of contractile muscle protein and the regulation of its rate of catabolism is also possible.

Methods are now available which enable the carrier for Duchenne muscular dystrophy to be detected with a 70–80% certainty. Our reported

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**Table Clinical data on seven Duchenne muscular dystrophy patients, with their 3-methylhistidine excretion and calculated myofibrillar protein catabolic rate compared to control values**

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Age (yr)</th>
<th>Weight (kg)</th>
<th>Creatinine excretion (g/day)</th>
<th>Muscle mass (kg)</th>
<th>Total muscle myofibrillar protein (kg)</th>
<th>3-MeHist creatinine (mg/day)</th>
<th>Mole ratio 3-MeHist creatinine (g/day)</th>
<th>Myofibrillar protein catabolism (g/kg body wt/day)</th>
<th>Myofibrillar protein catabolism (%/day)</th>
<th>Turnover myofibrillar protein (%/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17</td>
<td>53.2</td>
<td>0.363</td>
<td>7.26</td>
<td>0.94</td>
<td>24.3</td>
<td>0.045</td>
<td>53.2</td>
<td>1</td>
<td>5.66</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>60.5</td>
<td>0.209</td>
<td>4.18</td>
<td>0.54</td>
<td>16.9</td>
<td>0.054</td>
<td>37.0</td>
<td>0.61</td>
<td>6.85</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>57.0</td>
<td>0.234</td>
<td>4.68</td>
<td>0.61</td>
<td>22.3</td>
<td>0.064</td>
<td>48.8</td>
<td>0.86</td>
<td>8.00</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>65.5</td>
<td>0.231</td>
<td>4.62</td>
<td>0.60</td>
<td>8.0</td>
<td>0.023</td>
<td>17.5</td>
<td>0.27</td>
<td>2.92</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>41.8</td>
<td>0.146</td>
<td>2.92</td>
<td>0.38</td>
<td>11.7</td>
<td>0.054</td>
<td>25.6</td>
<td>0.61</td>
<td>6.73</td>
</tr>
<tr>
<td>6</td>
<td>15</td>
<td>74.8</td>
<td>0.189</td>
<td>3.78</td>
<td>0.49</td>
<td>15.4</td>
<td>0.055</td>
<td>33.7</td>
<td>0.45</td>
<td>6.88</td>
</tr>
<tr>
<td>7</td>
<td>19</td>
<td>65.9</td>
<td>0.227</td>
<td>4.54</td>
<td>0.59</td>
<td>37.9</td>
<td>0.111</td>
<td>82.9</td>
<td>1.26</td>
<td>14.05</td>
</tr>
</tbody>
</table>

Mean and range of values:

| Control mean and range of values N = 7 | 39.6 (32.8–49.7) | 0.018 (0.015–0.020) | 86.7 (71.8–108.7) | 1.28 (0.98–1.54) | 2.21 (1.87–2.46) |

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Additional references:


