Increased turnover of muscle contractile proteins in Duchenne muscular dystrophy as assessed by 3-methylhistidine and creatinine excretion

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Summary

1. Myofibrillar protein degradation has been measured in patients with Duchenne muscular dystrophy, normal boys, adult males and Duchenne carriers by the rate of 3-methylhistidine excretion after transfer of subjects to a meat-free diet.

2. Although absolute rates of protein breakdown are lower in Duchenne patients, expression of the data to allow for differences in muscle mass gives fractional degradation rates 2-3 times higher than in age-matched controls.

3. Fractional rates of muscle protein synthesis are increased in the Duchenne patients to almost the same extent as protein breakdown.

4. Rates of muscle protein breakdown in obligate and presumed carriers of the Duchenne gene are not different from controls.

Key words: creatinine, Duchene muscular dystrophy, 3-methylhistidine, protein degradation.

Introduction

Duchenne muscular dystrophy is usually diagnosed before 5 years of age, and progresses with increase in muscle weakness, decrease in muscle mass in relation to body weight and death frequently before age 20 years (Walton & Gardner-Medwin, 1974; Rowland, 1976). Although the sex-linked recessive inheritance is clearly understood, neither the nature of the genetic defect nor its localization in muscle cells has been resolved (Rowland, 1976). Notwithstanding this lack of information on the aetiology of the disease, the failure to accumulate contractile tissue is surely due to muscle protein degradation exceeding protein synthesis. An increase in protein synthesis observed in biopsy samples seems predominantly due to collagen synthesis, since the synthesis of other proteins, including the contractile proteins, is depressed (Ionasescu, 1975).

The excretion rate of 3-methylhistidine has been described as an index of myosin plus actin degradation (Johnson, Harris & Perry, 1967; Young, Alexis, Baliga, Munro & Muecke, 1972; Young, Haverberg, Bilmazes & Munro, 1973; Long, Haverberg, Young, Kinney, Munro & Geiger, 1975). With the exception of myosin from heart and red muscle fibres a single histidine residue is fully methylated in each of these contractile proteins (Johnson et al., 1967; Huszar & Elzinga, 1972) and on proteolysis the 3-methylhistidine is quantitatively excreted since it is not metabolized in the body (Young et al., 1972, 1973). We have now used 3-methylhistidine excretion to quantify rates of protein degradation and synthesis in patients with Duchenne muscular dystrophy. Rates are compared with those occurring in age-matched control subjects and with those of carriers of the Duchenne gene.
Methods

Patients and dietary restriction

Twenty boys aged 4-18 years (mean 11.3 years) with Duchenne dystrophy were used in the study. They had been diagnosed as a result of progressive muscle weakness, elevated blood activities of creatine kinase, electromyograms characteristic of myopathy and muscle biopsies indicative of the disease. Their average weight was 31.1 kg. Nine were ambulant at the time of the measurements. Boys used as controls had no indications of muscle disease and had an average age of 11.4 years and average weight of 38.6 kg.

In addition, nine normal adult males (average age 34 years), 14 adult females (average age 32 years) and 15 mothers of Duchenne patients (average age 34 years) were studied.

All subjects ate a diet devoid of any source of muscle protein (termed 'meat-free'). On days 4 and 5 of this regimen, two 24 h urine collections were taken with thymol as a preservative (Tomas, Ballard & Pope, 1979) since we had shown that 3 days of a muscle-free diet was needed to prevent the diet contributing to 3-methylhistidine excretion.

Analytical measurements

Methods for the analysis of 3-methylhistidine and creatinine in urine are described in the accompanying paper, together with details of the determinations of 3-methylhistidine and total protein in human muscle (Tomas et al., 1979). Values for these last two measurements on the left vastus lateralis muscle obtained at autopsy from six children aged between 4 and 13 years were 3.59 ± 0.07 (SEM) μmol of 3-methylhistidine/g of muscle protein and 200 ± 10 (SEM) mg of protein/g fresh weight of muscle (Tomas et al., 1979).

Values are given as mean ± SEM.

Results

Estimation of muscle mass

To calculate rates of muscle protein turnover relative to the total muscle protein (fractional rates) we require measurements or estimates of the amount of muscle protein in normal boys and in patients with Duchenne muscular dystrophy at different stages during the progression of the disease. Since direct measurements are not available we have taken the excretion of 1 g (8.85 mmol) of creatinine/day as being derived from 20 kg of muscle, a value obtained from normal growing boys (Graystone, 1968). For patients with Duchenne muscular dystrophy the excretion of 62 μmol of creatinine day⁻¹ kg⁻¹ body weight in 5-7 year olds (Fig. 1) corresponds to a muscle mass that is 14% of the body weight. By age 17 the creatinine excretion had dropped to 25 μmol day⁻¹ kg⁻¹, indicating that only 6% of the body weight is muscle. Although the normal boys showed considerable variability in creatinine excretion per unit body weight (Fig. 1), their mean excretion rate of 185 μmol day⁻¹ kg⁻¹ is much higher than in the patients with dystrophy and would correspond to 42% of the body weight being muscle (Graystone, 1968). Slightly higher values were found in the adults (Fig. 1).

The average rates of creatinine excretion in the patients with dystrophy and normal boys were 1.25 mmol/day and 7.3 mmol/day respectively (Table 1). From these values, the relationship between creatinine and muscle mass (Graystone,

![Figure 1](attachment:image.png)

**FIG. 1.** Excretion of creatinine by normal and Duchenne dystrophic subjects. Values shown are the creatinine excretion rates in μmol day⁻¹ kg⁻¹ body wt. and are the average of two sequential, 24 h urine collections on days 4 and 5 of a muscle-free diet. ○, Normal subjects; O, dystrophic patients. The bar represents the range of values for nine adult males (A).

<table>
<thead>
<tr>
<th>Table 1. Estimation of muscle mass</th>
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<tbody>
<tr>
<td>Details of the measurements in 11 normal and 20 dystrophic boys and the calculations are given in the text.</td>
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<tr>
<th></th>
<th>Normal</th>
<th>Dystrophic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine excretion (mmol/day)</td>
<td>7.30</td>
<td>1.25</td>
</tr>
<tr>
<td>Total muscle protein (kg)</td>
<td>3.30</td>
<td>0.56</td>
</tr>
<tr>
<td>Muscle protein accumulation (g/day)</td>
<td>0.9</td>
<td>0.0</td>
</tr>
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Protein turnover in muscular dystrophy

TABLE 2. Muscle protein metabolism in normal and dystrophic boys

Details of the calculations are given in the text. Values are means ± SEM for data on 11 normal and 20 dystrophic boys.

<table>
<thead>
<tr>
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<th>Normal</th>
<th>Dystrophic</th>
</tr>
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<tbody>
<tr>
<td>Urinary excretion of 3-methylhistidine (µmol/day)</td>
<td>172 ± 19</td>
<td>78.0 ± 5.5</td>
</tr>
<tr>
<td>Urinary excretion of 3-methylhistidine (µmol day⁻¹ kg⁻¹)</td>
<td>4.52 ± 0.20</td>
<td>2.62 ± 0.21</td>
</tr>
<tr>
<td>3-Methylhistidine/creatinine molar ratio</td>
<td>0.024 ± 0.0006</td>
<td>0.058 ± 0.0015</td>
</tr>
<tr>
<td>Muscle protein catabolism (g/day)</td>
<td>47.9</td>
<td>21.7</td>
</tr>
<tr>
<td>Fractional rate of protein catabolism (%/day)</td>
<td>1.48 ± 0.03</td>
<td>3.59 ± 0.09</td>
</tr>
<tr>
<td>Muscle protein synthesis (g/day)</td>
<td>48.8</td>
<td>21.7</td>
</tr>
<tr>
<td>Fractional rates of protein synthesis (%/day)</td>
<td>1.51</td>
<td>3.59</td>
</tr>
</tbody>
</table>

1968) and the protein content of muscle (200 mg/g; Tomas et al., 1979) the total amount of muscle protein was calculated. The amounts of muscle protein were 3-30 kg in the normal boys and 0.56 kg in the patients with dystrophy (Table 1).

The total daily excretion of creatinine in the dystrophic boys does not change with age, implying no increase in muscle mass (Table 1). In normal boys, on the other hand, growth tables show an increase in body weight averaging 4·1 kg/year between ages 8½ and 14½ (Heimendinger, 1970). Since muscle comprises 42% of the body weight in the normal boys and the protein content of muscle is 200 mg/g (Tomas et al., 1979), normal boys are accumulating muscle protein at the rate of 0.34 kg/year, or about 0.9 g/day (Table 1).

Muscle protein catabolism

Under the dietary regimen used in the present experiments the 3-methylhistidine excreted in urine will be almost totally derived from the catabolism of endogenous actin and myosin. 3-Methylhistidine excretion rates average 172 µmol/day or 4·52 µmol day⁻¹ kg⁻¹ for the normal boys, and 78 µmol/day or 2.62 µmol day⁻¹ kg⁻¹ for the patients with dystrophy (Table 2).

The expression of 3-methylhistidine excretion rates by either of the above methods takes no account of the proportion of muscle tissue in the body. Accordingly, there is considerable variation between individuals, especially when boys of different ages are compared. However, when results are expressed per unit of creatinine excreted, an allowance is made for differences in muscle content. Thus the normal boys excrete between 0.021 and 0.027 µmol of 3-methylhistidine/µmol of creatinine, with mean 0.024. The dystrophic boys have a much greater rate of 3-methylhistidine excretion on this basis, with a molar ratio ranging between 0.045 and 0.073 and a mean of 0.058 (Table 2, Fig. 2).

The average degradation rate of muscle protein (g/day) can be obtained by dividing the 3-methylhistidine excretion per day by the 3-methylhistidine content of muscle protein (3·59 µmol/g; Tomas et al., 1979). In this way mean values of 47·9 g/day in normal boys and 21.7 g/day in the patients with dystrophy are obtained (Table 2).

The fraction of contractile proteins degraded each day can be calculated from the expression:

\[
\% \text{ Protein degraded per day} = \frac{\mu\text{mol of 3-methylhistidine excreted per day} \times 100}{\mu\text{mol of 3-methylhistidine in muscle protein}}
\]

The denominator of this equation is the product of the 3-methylhistidine content in muscle protein and per g of creatinine for nine adult males gave a mean value of 0.018 µmol/g (Fig. 2).
FIG. 3. 3-Methylhistidine excretion in mothers of Duchenne dystrophic boys and in normal adult females. Mothers are grouped as obligate, presumed or possible carriers according to criteria outlined in the text. Values are the 3-methylhistidine to creatinine molar excretion ratios and are the average of duplicate analyses on sequential 24 h urine collections on days 4 and 5 of a muscle-free diet. Horizontal lines indicate the mean of each group.

the amount of muscle protein, which in turn is calculated from the creatinine excretion rate (1 mg of creatinine excreted is equivalent to 20 g of muscle) and the protein content of muscle (200 mg/g). The percentage of myofibrillar protein degraded each day is then 1.48 ± 0.03% for the normal boys and 3.59 ± 0.09% for the patients with dystrophy (Table 2).

Muscle protein synthesis

Rates of protein synthesis are the sum of the protein accumulation and protein catabolism rates. By substitution of the daily rates of protein accumulation and degradation, values of 48.8 g/day in normal boys and 21.7 g/day in dystrophy patients are obtained. Similarly, the fractional rates of protein synthesis are 1.51%/day for normal boys and 3.59%/day for the patients with dystrophy (Table 2).

Possible detection of Duchenne carriers

The small amount of variability in the 3-methylhistidine to creatinine molar ratio among normal individuals coupled with the large increase in this variable in patients with Duchenne muscular dystrophy suggests a method for screening carriers of the recessive gene. Accordingly, rates of 3-methylhistidine excretion per g of creatinine were measured in 15 mothers of affected boys and 14 adult females with no family history of muscle disease. For this comparison the mothers of affected boys were grouped as (a) obligate carriers, if they had elevated serum creatine phosphokinase activities plus either two sons with Duchenne dystrophy or another affected male on the maternal side, (b) presumed carriers, if they had elevated serum creatine phosphokinase activities, or (c) possible carriers if their creatine phosphokinase activities were normal. By this classification the 3-methylhistidine to creatinine molar excretion ratio averaged 0.018 in the control group, 0.021 in four possible carriers, 0.020 in six presumed carriers and 0.018 in four obligate carriers (Fig. 3).

Discussion

Possible errors in the assessment of muscle mass and 3-methylhistidine pool size

The main assumptions made in this study are that (1) creatinine excretion can be used as an index of muscle mass with a production rate of 8.85 mmol/day being equivalent to 20 kg of muscle in both normal and dystrophic subjects, and (2) the 3-methylhistidine content of 3.59 μmol/g of muscle protein in normal human muscle (Tomas et al., 1979) can also be applied to the dystrophic muscle.

The validity of the relationship between creatinine excretion and muscle mass has not been tested in dystrophic humans nor in the animal models of the disease. However, the very low amounts of muscle protein we have calculated are qualitatively consistent with clinical evaluation of muscle content (Walton & Gardner-Medwin, 1974). Indeed the amount of muscle present could actually be less than calculated, because the increased creatinine excretion in dystrophic patients will result in an overestimation of muscle mass if any creatine is converted into creatinine by non-enzymic reactions in the urine (Benedict, Kalinsky, Scarrone, Wertheim & Stetten, 1955). Against this trend to overestimate muscle mass is the finding that concentrations of creatine phosphate, the normal source of creatinine, are lower than normal in biopsy samples of muscle from patients with Duchenne muscular dystrophy (Stengl-Rutkowski & Barthelmai, 1973). A lowered concentration of creatine phosphate probably results from the reduced actomyosin content of dystrophic muscle (Schapira, Dreyfus, Schapira & Kruh, 1955; Simon, Gross & Lessell, 1962), and indeed it is likely that creatinine excretion is a more accurate index of the mass of contractile elements (which also contains the 3-methylhistidine residues) than of total muscle.
In the absence of definitive measurements it is therefore reasonable to use creatinine excretion as an index of 'functional' muscle mass in the dystrophic boys in the same manner as for normal boys.

The large increase in fractional protein breakdown found in the dystrophic boys could be an overestimate if the 3-methylhistidine content of their muscle was higher than normal. Although muscle autopsy or biopsy samples from Duchenne patients have not been analysed for 3-methylhistidine content, an increased amount/mol of actin or myosin is unlikely because most of the myosin and all of the actin, which contains 70–80% of the 3-methylhistidine, show complete methylation of single histidine residues in normal muscle (Johnson et al., 1967; Huszar & Elzinga, 1972). Physical and chemical studies do not indicate any differences in myofibrillar protein properties between normal and dystrophic muscle such as may be expected if additional methylation occurred (Rowland, 1976). Further, the 3-methylhistidine content of dystrophic mouse muscle is lower than normal (1.87 μmol/g of protein versus 3.44 μmol/g of protein from normal mouse muscle; F. M. Tomas & F. J. Ballard, unpublished data), in accord with a lower proportion of myofibrillar protein in this condition (Simon et al., 1962).

**Increased protein turnover in Duchenne dystrophy**

The 3-methylhistidine excretion data reported in Table 2 and Fig. 2 show 2–3-fold increases in the fractional rate of muscle protein catabolism in the dystrophic patients, as compared with age-matched controls, confirming the observations of McKeran, Halliday & Purkiss (1977), published after the present work had been completed. Much greater variability in 3-methylhistidine excretion was found by McKeran et al. (1977) than in our study, with a 3-methylhistidine to creatinine molar ratio of between 0.023 and 0.111 in seven patients (compare with Fig. 2). Although we cannot explain the low excretion rate found in one patient, variability at the high end of the range was probably caused by meat in the patients’ diet (see Tomas et al., 1979). McKeran et al. (1977) calculated an average fractional degradation rate for myofibrillar proteins of 7.3% and 2.21% per day in dystrophic and normal subjects respectively. These rates are twice as high as in our study, due to use of a much lower 3-methylhistidine content in muscle (1.76 μmol/g of muscle protein; Asatoor & Armstrong, 1967). Our values of 3-methylhistidine content in muscle samples from children (Tomas et al., 1979) are twice those reported by Asatoor & Armstrong (1967).

The increased activities of lysosomal proteases in muscle from patients with Duchenne muscular dystrophy (Kar & Pearson, 1976, 1977) are consistent with the greater rate of muscle protein catabolism found both in the present study and by McKeran et al. (1977). Also the genetic dystrophies in mice and chickens are accompanied by increased activities of proteolytic enzymes (Simon et al., 1962; Iodice, Chin, Perker & Weinstock, 1972; Rourke, 1975).

The calculations in Table 2 show that muscle protein synthesis in the dystrophic boys is increased to almost the same extent as protein breakdown. If synthesis was not increased, muscle wasting would be extremely rapid with a net loss of about 2% per day. Accordingly, the depressed rate of myofibrillar protein synthesis reported in biopsy samples from Duchenne patients (Ionasescu, 1975) is probably an artifact from this technique in vitro. The situation in dystrophic mice and chickens is less clear, with both lower (Nihei, Tataryn & Filipenko, 1973) and higher (Simon et al., 1962; Rourke, 1975) rates of muscle protein synthesis being found.

The fractional rate of protein degradation in the dystrophic boys, although 2–3 times higher than age-matched controls, is comparable with the rate found in premature infants (Tomas et al., 1979). Perhaps dystrophy can be considered as a defect in development that is related to a continuing high rate of myofibrillar protein breakdown. Alternatively dystrophic infants may have an even higher protein turnover than normal infants, a property which could be used for early diagnosis of the syndrome.

**Possible uses of the 3-methylhistidine technique in the prevention or treatment of Duchenne dystrophy**

The large difference in fractional catabolism rates between normal and dystrophic subjects offers several approaches to the management of the disease. However, it is unlikely that carriers of the gene can be detected because the range of 3-methylhistidine to creatinine excretion rates found in obligate and presumed carriers falls within the spread of normal values (Fig. 3).

Treatment of Duchenne dystrophy by a reduction in protein degradation rates does, however, seem worthwhile. The extent of inhibition needed to
obtain a beneficial response is quite modest. Thus if protein degradation in the dystrophic boys could be reduced only 4% to 20-8 g/day, without changing the synthesis rate from 21·7 g/day, this would give a protein accumulation rate of 0-9 g/day, equal to that observed in normal growing boys (Tables 1 and 2). Any greater inhibition would permit catch-up growth. The increasing amount of information on compounds which reduce protein breakdown such as hormones, nutrients, weak bases and inhibitors of proteolytic enzymes suggests a fruitful approach to the treatment of Duchenne dystrophy (Goldberg & St. John, 1976; Ballard, 1977). Encouraging results have already been reported with isolated dystrophic muscle (Iodice, 1976; McGowan, Shafiq & Stracher, 1976) and while negative responses to some proteolytic inhibitors have been reported for dystrophic mice (Enomoto & Bradley, 1977) other compounds may prove successful.

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References


