Biochem. J. (1990) 268, 795–797 (Printed in Great Britain)

Protein turnover is elevated in muscle of *mdx* mice *in vivo*

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mdx mice lack the protein dystrophin, the absence of which causes Duchenne muscular dystrophy in humans. To examine how *mdx* mice maintain muscle mass despite dystrophin deficiency, we measured protein turnover rates in muscles of *mdx* and wild-type (C57BL/10) mice *in vivo*. At all ages studied, rates of muscle protein synthesis and degradation were higher in *mdx* than in C57BL/10 mice.

INTRODUCTION

Dystrophin, the protein product of the gene which is defective in Duchenne muscular dystrophy [1], is not expressed in the muscles of *mdx* mice [2]. However, these mice show few signs of the human disease: although histological abnormalities of muscle and elevated activities of muscle enzymes in serum are observed [3], the deficit does not adversely affect muscle growth or function [4]. To investigate the mechanism through which *mdx* mice preserve muscle mass, we have measured rates of protein turnover in *mdx* and control (C57BL/10) mice of different ages.

The rate of protein deposition or loss ($k_{dep.}$) in a tissue is determined by the relative rates of protein synthesis (k_s) and protein degradation (k_d) according to the equation $k_{dep.} = k_s - k_d$ [5]. We examined the rates of these processes in muscle of *mdx* and wild-type (C57BL/10) mice by direct determination of $k_{dep.}$ and k_s, with k_d being calculated by difference. Liver k_s and muscle RNA concentration were also measured.

EXPERIMENTAL

Materials

L-[2,3-^{3}H]Phenylalanine was purchased from Amersham International, Amersham, Bucks., U.K. All other chemicals and biochemicals were purchased from Sigma Chemical Co., Poole, Dorset, U.K., or BDH, Poole, Dorset, U.K. Breeding pairs of *mdx* and C57BL/10 mice (wild-type) (derived from the colony originally identified by Bullfield *et al.* on the basis of elevated serum pyruvate kinase activities [3]) were generously given by Dr. T. Partridge. Mice of the C57BL/10 and *mdx* strains were maintained on a 12 h light/12 h dark cycle with food and water available *ad libitum*.

Methods

Tissue k_s was determined by an adaptation [6] of the method of Garlick *et al.* [7]. Briefly, mice were injected intraperitoneally (2 ml/100 g body weight) with a solution consisting of 150 mM-[^{3}H]phenylalanine (75 μCi/ml) in 150 mM-NaCl. The animals were killed 15 min after injection; samples of liver and the gastrocnemius/plantaris/soleus muscle group from one hind limb were rapidly removed and cooled then frozen in liquid N_2 before measurement of the specific radioactivities of free and protein-bound phenylalanine.

The same muscle group was then carefully dissected from the contralateral limb and its protein content assayed [8]. To determine muscle $k_{dep.}$ the protein content of these muscles was measured in animals which were either 3 days older (in the case of 3-week-old mice) or 6–10 days older (all other experiments) than those in which muscle k_s was assayed. Muscle $k_{dep.}$ was expressed as the percentage change in protein content per day. Tissue protein synthetic rates were calculated from the equation described by Jepson *et al.* [6]. The RNA concentrations of acidified supernatants from tissue KOH digests were measured as described by Munro & Fleck [9] using the equation: RNA concentration (μg/ml) $= 35.8A_{260} - 15.16A_{232}$. Muscle k_d was calculated from the difference between $k_{dep.}$ and k_s.

RESULTS

The protein content of the hind limb muscle group in animals aged 7 weeks or older was significantly higher in *mdx* mice than in controls (Fig. 1), confirming that protein accretion is not defective in *mdx* mice. Fig. 2 shows that, with increasing age, k_s, k_d and $k_{dep.}$ of *mdx* and control mice decreased, a finding which is in accord with previous studies in the rat [10]. However, at all ages muscle k_s and k_d were greater in *mdx* mice than in controls; in muscle of growing animals, $k_{dep.}$ was also elevated in *mdx* mice.

Measurements of muscle RNA concentrations can provide some insight into the mechanism through which control of muscle k_s is exerted [11]. More than 90% of RNA is ribosomal [12], and RNA concentration therefore provides an index of tissue ribosome concentration. Thus by calculating the amount of protein synthesized/day per unit of RNA, an indication of the efficiency of translation, i.e. the rate of peptide synthesis per ribosome, is obtained. Table 1 shows that the elevated protein synthetic rate observed in *mdx* mouse muscle was mediated through alterations in tissue ribosome concentration, with translational efficiency being unchanged.

A further notable finding of the present study was that liver protein synthetic rates were similar in *mdx* and control mice at all ages studied (Table 2). Dystrophin is not expressed in livers of normal mice or humans [2], suggesting that the increased protein turnover rate observed in *mdx* muscle was the specific consequence of dystrophin deficiency in this tissue.

DISCUSSION

In growing animals, the rate of muscle protein deposition ($k_{dep.}$, i.e. growth) is dependent on the extent to which k_s exceeds k_d. The muscles of growing *mdx* mice showed elevations of both k_s and k_d; furthermore, the difference between k_s and k_d was greater in *mdx* than in C57BL/10 mice, with the consequence that *mdx* muscle $k_{dep.}$ was also increased.

Abbreviations used: k_s, fractional rate of protein synthesis; k_d, fractional rate of protein degradation; $k_{dep.}$, fractional rate of protein deposition.
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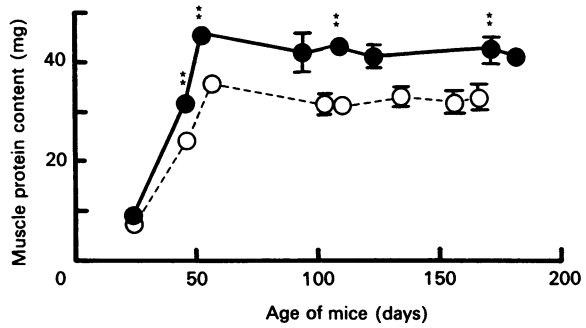

Fig. 1. Protein content of hind limb muscle group in *mdx* and control mice

The muscle group consisting of gastrocnemius, soleus and plantaris was carefully removed from one hind limb of C57BL/10 (○) and *mdx* (●) mice of different ages and its protein content was determined. Values are means ± S.D., $n = 3$–5.

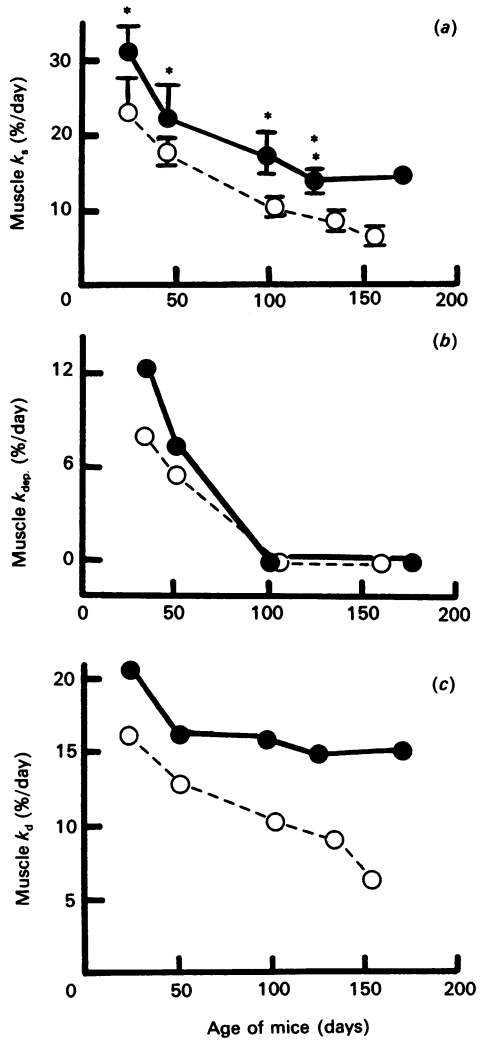

Fig. 2. Rates of hind limb muscle protein synthesis (*a*), protein deposition (*b*) and protein degradation (*c*) in C57BL/10 (○) and *mdx* (●) mice of different ages

Further details are given in the text. Protein synthetic rates are expressed as means ± S.D.; *($P < 0.05$) and **($P < 0.001$) indicate statistically significant differences between C57BL/10 and *mdx* mice of similar ages. Protein deposition and degradation rates are expressed as means; $n = 3$–5.

Table 1. Hind limb muscle RNA concentrations and translational efficiencies in C57BL/10 and *mdx* mice

Further details are given in the text. Values are means ± S.D. *($P < 0.05$) and **($P < 0.001$) indicate statistically significant differences in muscle RNA concentration in *mdx* mice compared with C57BL/10 mice of similar ages.

Age (days)	Muscle RNA concentration (mg/g of protein)		Translational efficiency (g of protein synthesized/day per g of RNA)	
	C57BL/10	*mdx*	C57BL/10	*mdx*
24	16.2±1.7	19.5±0.5*	14.6±4.0	16.1±2.1
46	8.9±1.8	13.2±0.6**	18.0±3.0	17.0±3.2
99–102	8.4±0.5	11.8±1.2**	12.0±1.3	14.9±2.7
123–134	6.2±0.4	12.4±0.9**	14.1±2.2	12.4±1.6
156–171	6.2±1.2	8.8±1.7**	10.9±3.6	18.2±5.0

Table 2. Liver protein synthetic rates in C57BL/10 and *mdx* mice of different ages

Further details are given in the text. Values are means ± S.D. There were no statistically significant differences between C57BL/10 and *mdx* mice of similar ages

Age (days)	Liver k_s (%/day)	
	C57BL/10	*mdx*
24	90±12	97±19
46	81±15	81±11
99–102	68±7	61±12
123–134	59±12	53±7
156–171	55±11	49±7

One possible factor in causing the increased k_s might have been the infiltration of *mdx* muscles with non-muscle cells which had an inherently higher rate of protein turnover. However, this seems unlikely, because k_s was increased to a roughly equal extent in both contractile and non-contractile protein fractions prepared from *mdx* muscles [13]. The contractile protein content of non-muscle cells would be negligible.

It seems likely that the elevated protein turnover observed in *mdx* mouse muscles is a consequence of their being subject to a continued process of damage and repair, a suggestion which is supported by the histological observation of numerous degenerating and regenerating fibres in these muscles [14]. Current evidence indicates that the increased intracellular Ca^{2+} content of *mdx* muscle fibres [14,15] may initiate the myopathic events. It has been demonstrated that the Ca^{2+} ionophore A23187 causes increased proteolysis [16] and induces damage to muscle of normal mice and rats [17] through mechanisms which may involve activation of phospholipase enzymes [18].

Experiments *in vitro* which examined rates of tyrosine release from *mdx* and C57BL/10 muscles [15] may provide some support for this suggestion. When incubated at 1.8 mM extracellular Ca^{2+}, the tyrosine release rates and intracellular Ca^{2+} concentrations of *mdx* muscles were greater than those of C57BL/10 muscles. However, during incubation at 0.18 mM extracellular Ca^{2+}, both tyrosine release rates and intracellular Ca^{2+} concentrations of *mdx* and C57BL/10 muscles were equal. Although these muscle

preparations were in negative nitrogen balance and thus unphysiological, the results are consistent with the hypothesis that elevated intracellular Ca^{2+} is linked to increased k_d in *mdx* mice. The present study suggests that, *in vivo*, *mdx* muscle nitrogen balance is maintained despite increased k_d through elevation of k_s. The increased tyrosine release from *mdx* muscle *in vitro* may reflect the fact that muscle k_s is compromised in incubated systems [19]; thus k_s cannot be raised to compensate for increased k_d.

If the degenerative influence of dystrophin deficiency may be overcome by elevation of muscle k_s, it is of importance to discover why this protective strategy is available to mice but not to humans. Indeed, in boys suffering from Duchenne muscular dystrophy, muscle k_s is depressed [20]. It is possible that smaller animals have a greater regenerative capacity, since there is an inverse relationship between k_s and body weight [21], and it is of relevance in this context that the dystrophin-deficient dog experiences muscle wasting and necrosis [22]. A second possibility is that the increased gravitational forces and stresses to which muscle of larger animals is subjected may play a role [23].

We thank the Muscular Dystrophy Group of Great Britain and Northern Ireland for their financial support and Dr. Malcolm Jackson for many useful discussions. The technical assistance of Miss A. McArdle, Miss A. Swift and the staff of the Royal Liverpool Hospital Animal Unit is also acknowledged. We are grateful to Miss L. Nichol for her secretarial assistance.

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Received 22 November 1989/10 April 1990; accepted 25 April 1990