Characteristics of skeletal muscle growth and protein turnover in a fast-growing rat strain

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1. Protein turnover and muscle composition has been studied in rat skeletal muscle throughout development in a relatively-fast-growing rat strain.

2. Muscle growth involved an increase in the total DNA and in the DNA-unit size as indicated by protein: DNA. As a result of the latter increase together with no change in RNA: DNA, the RNA concentration fell throughout development.

3. Rates of protein synthesis measured in vivo by the continuous intravenous infusion method fell throughout development from 15·6%/d at 25 d to 4·46%/d at 320 d, and these changes reflected mainly the fall in RNA concentration, since there was no marked change in the rate of protein synthesis per unit RNA.

4. The rate of protein degradation, measured as the difference between rates of protein synthesis and growth, fell from 9·82%/d at 25 d to 4·46%/d at 320 d.

5. When these changes in protein turnover throughout development are compared with measurements made previously in a slow-growing strain it would appear that the faster growth was achieved as a result of increased efficiency of protein synthesis (defined as net synthesis: over-all synthesis) and this occurred mainly because of lower rates of protein degradation.

The continuous turnover of muscle proteins means that the growth of muscle, like that of most tissues, can be regulated through changes in the rates of both protein synthesis and degradation. Protein synthesis in muscle has been shown to be an important site of regulation, being particularly sensitive to a wide range of nutritional and hormonal factors (e.g. Millward & Waterlow, 1978) as well as the nature and extent of contractile activity (Bates *et al.* 1980; Laurent & Millward, 1980). Protein degradation also appears to be an important regulatory site but in this instance changes are often paradoxical. Thus in some instances increases in the rate of degradation occur during muscle anabolism and decreases can occur during muscle catabolism (see Waterlow *et al.* 1978; Millward *et al.* 1980). Thus the changes in protein degradation appear to be counter productive and necessitate very marked changes in protein synthesis to achieve the appropriate net response. A particularly important example is the pattern of changes in protein turnover during development. In this instance protein degradation rates are high in young rapidly-growing rat muscles and fall with age (Millward *et al.* 1975), and this appears to be a general interspecies feature of muscle growth and development (Millward, 1978, 1980*a*). In the present study we have examined the developmental changes in protein turnover in muscle in a particularly fast-growing strain of rat (CFY albino) to see the extent to which the developmental changes in skeletal muscle protein turnover differ from those we have previously described for a slow-growing strain (Millward *et al.* 1975) and to determine the mechanism of the faster growth. A preliminary account of these results has been presented previously (Bates & Millward, 1978).

EXPERIMENTAL

The rats studied were an albino strain (CFY) originally obtained from Anglia Laboratory Animals but subsequently bred in the Clinical Nutrition and Metabolism Unit. Rats were weaned at 4 weeks of age and measurements were made on groups of male litter-mates which

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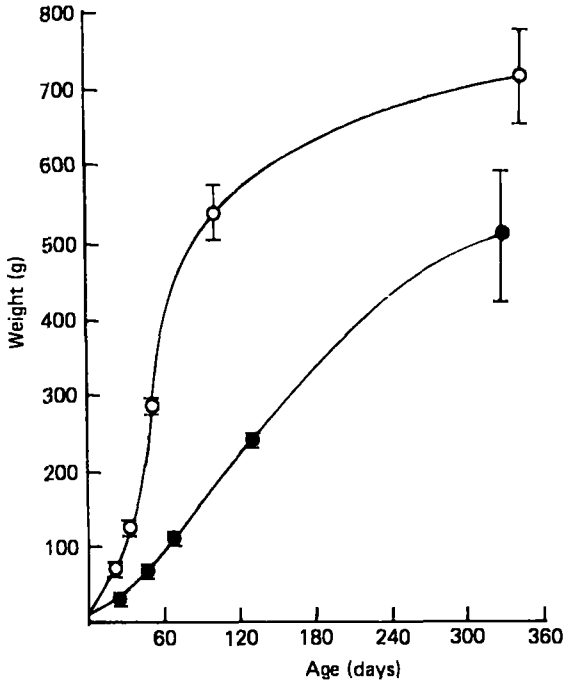

Fig. 1. Growth curve of male CFY albino strain (○) and male hooded strain described previously (●) (Millward *et al.* 1975). Points represent means ± 1 standard deviation.

were all fed on a standard laboratory diet (Oxoid) *ad lib.* Measurements were made of the rates of protein synthesis and degradation in the combined gastrocnemius and quadriceps muscles and the composition of these muscles in terms of RNA, DNA and non-collagen protein. The rate of protein synthesis was measured by the continuous intravenous infusion of C^{14}-labelled tyrosine as previously described (Millward *et al.* 1975). The measurements of the degradation rate were made by observing the growth rate of each rat over the few days preceeding the infusion and assuming that this rate was obtained during the infusion. The degradation rate was then calculated by subtracting the growth rate from the measured rate of protein synthesis. Inherent in this calculation is the assumption that over the small time interval at which the growth of the rat was measured the growth of the protein mass in the muscle was proportional to that of the whole body. Also inherent in this calculation is the assumption that the measured rate of protein synthesis, which was made between 09.00 and 15.00 hours was a reasonable approximation of the average rate of the whole day (see Waterlow *et al.* 1978).

The amounts of RNA, DNA and non-collagen protein in the combined gastrocnemius and quadriceps muscles were determined on muscle taken from the rat at the end of the 6 h infusion which were analysed according to methods described previously (Millward *et al.* 1974).

RESULTS

The pattern of growth of male rats of the CFY strain is shown in Fig. 1. The growth of a hooded strain which we have previously studied is also shown for comparison. It is obvious that CFY rats grow more rapidly and achieve a larger ultimate body-weight than the hooded rats. Although we have not analysed the body composition of these rats the measurements

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Table 1. *Composition of combined gastrocnemius and quadriceps muscle throughout development*

(Mean values and 1 standard deviation for five to six measurements)

Age (d)	Body-wt (g)		Muscle wt (% body-wt)		Total DNA (mg)		Protein:DNA		RNA:DNA	
	Mean	1 SD	Mean	1 SD	Mean	1 SD	Mean	1 SD	Mean	1 SD
25	75	2·6	0·96	0·06	0·70	0·08	167	15	2·07	0·17
32	129	10	1·02	0·06	1·26	0·14	180	13	1·71	0·09
52	289	19	1·17	0·03	1·75	0·08	290	22	2·11	0·09
101	546	45	1·11	0·05	2·43	0·38	400	42	1·79	0·28
320	716	60	1·00	0·06	2·69	0·19	452	29	1·88	0·35

Table 2. *Labelling of free tyrosine in the plasma* (S_p) *and intracellular pool* (S_i), *and calculated rates of protein synthesis* (K_s), *rate of protein growth* (K_g) *and rate of protein degradation* (K_d) *in muscle throughout development*

(Mean values and 1 standard deviation for five to six measurements)

Age (d)	$S_i:S_p$		K_s (%/d)		K_g (%/d)		K_d (%/d)	
	Mean	1 SD	Mean	1 SD	Mean	1 SD	Mean	1 SD
25	0·96	0·17	15·6	1·3	6·31	0·56	9·82	0·86
32	0·93	0·11	15·2	2·9	5·61	0·30	9·54	2·73
52	0·72	0·09	7·34	0·56	2·98	0·05	4·36	0·60
101	0·68	0·07	5·16	0·05	1·08	0·03	4·09	0·51
320	0·70	0·08	4·46	0·05	ND		4·46	0·05

ND, not detectable.

of the mass of the combined gastrocnemius and quadriceps muscles (Table 1) as a proportion of body-weight follows a similar pattern to that of the hooded rats previously described (Millward *et al.* 1975). Although in the heaviest rats muscle mass as a proportion of body-weight was reduced compared with younger lighter rats, the difference was small, indicating that if there was increased fat deposition in the heavier rats, it was not extensive.

The muscle growth involved increases in both total DNA and in protein: DNA as previously demonstrated (Millward *et al.* 1975). However, RNA:DNA did not markedly change throughout development.

The measurements of protein synthesis are shown in Table 2. At the end of the infusion the intracellular free tyrosine labelling was very similar to that of plasma in the two youngest groups but was approximately 30% lower in the older groups. Since the plateau labelling of the intracellular pool compared with the plasma is determined by the relative rates of exchange of the intracellular tyrosin between the protein pool (a rate which falls with age) and the extracellular pool, this indicates that there was a fall in the rate of tyrosine transport into and out of the muscle with age. The rates of protein synthesis (K_s) calculated from the ratio, tissue free:protein-bound tyrosine labelling at the end of the infusions fell from 15.6%/d in the youngest rats to 4·46%/d in the oldest rats. When the growth rates of the muscle protein mass were compared with the over-all rates of protein synthesis it is obvious

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Table 3. *Capacity for protein synthesis and RNA activity in skeletal muscle throughout development*

(Mean values and 1 standard deviation for five to six measurements)

Age (d)	RNA/protein ($\times 10^3$) Mean	1 SD	RNA activity (g protein synthesized/d per g RNA Mean	1 SD
25	12·4	0·52	12·6	1·3
32	9·57	0·60	14·7	2·2
52	7·28	0·23	10·1	0·7
101	4·50	0·60	11·6	1·3
320	4·13	0·66	10·9	1·5

that degradation rates were 9·82%/d at 25 d falling to 4·46%/d at 1 year of age. The rate of protein synthesis can be compared with the RNA concentration enabling protein synthesis to be expressed in terms of the capacity for protein synthesis, conveniently indicated by the RNA:protein value and the RNA activity (i.e. the rate of protein synthesis per unit RNA determined by dividing K_s by RNA:protein (Millward *et al.* 1973). As indicated in Table 3 the main factor responsible for the fall in the rate of protein synthesis with age was the fall in the capacity for protein synthesis. The RNA activity did not substantially change with age.

DISCUSSION

The males of the CFY rat strain examined in this study are characterized by a large ultimate body-weight and a more rapid growth rate than the hooded strain which we have previously studied (Millward *et al.* 1975). In fact the growth curve is similar to that described by Ross *et al.* (1976) for Charles River COBS CD rats. However, the mean life span of the CFY rats studied here is only approximately 1 year according to studies in this Department (E. Wheeler, personal communication), compared to 2 years for both the hooded rats (E. Wheeler, personal communication) and the COBS CD rats (Ross *et al.* 1976). These differences in body-weights and life-span suggest that it is difficult to compare the developmental changes in protein turnover between the two species. Whilst at weaning (1 month) the rate of protein synthesis and degradation were markedly lower for the CFY rats compared to hooded rats (Table 4), in rats of similar weight any differences were much less marked. We have no information as to whether smaller CFY rats would have exhibited synthesis rates as high as in the smallest hooded rats.

The fall in the rate of protein synthesis with age in this study was associated primarily with a fall in the RNA concentration (as indicated by RNA:protein, Table 3). The mechanism of this fall in RNA concentration appears to be an enlargement of the amount of cytoplasm per nucleus (i.e. indicated by the DNA unit size or protein:DNA, Table 1), whilst RNA:DNA stayed constant. Since the RNA activity did not change with age, the DNA activity, i.e. the rate of protein synthesis per unit DNA, did not alter with age. Such observations as this have led us to formulate a working hypothesis for the regulation of cell size (more strictly DNA-unit size as indicated by protein:DNA) in which, with a constant zero-order rate of protein synthesis (the DNA activity), cell size is determined by the first order degradation rate (Millward *et al.* 1977, Millward, 1980*a*). Thus in these terms, in the present results the fall in the degradation rate with age allows the enlargement of the muscle DNA-unit.

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Table 4. *The efficiency of muscle protein synthesis during growth: net protein synthesis expressed as a proportion of over-all protein synthesis in the rat*

Strain	Age (d)	Body-wt (g)	Protein synthesis (%/d)	Protein degradation (%/d)	Net synthesis (%/d)	Efficiency (net/over-all × 100)
CFY	25	75	15·6	9·8	6·3	40·4
	32	129	15·2	9·5	5·6	36·8
	52	289	7·3	4·4	3·0	41·1
	101	546	5·2	4·1	1·1	21·2
	320	716	4·5	4·5	—	—
Hooded*	23	37	28·6	22·5	6·27	21·9
	46	70	16·1	13·1	2·97	18·4
	65	116	11·5	9·8	1·71	14·4
	130	233	5·3	4·6	0·72	13·5
	330	511	4·9	4·9	—	—

* Results of Millward *et al.* (1975).

It should be possible to account for the faster growth rate of muscle protein in this strain in terms of a difference in the rates of muscle protein synthesis and degradation compared with our previous measurements of these processes in the hooded strain. There are several ways in which this can be done. The net synthesis rate (K_g) can be calculated as a proportion of total synthesis (K_s) which is one indication of the efficiency of muscle protein synthesis. This has been done for the present and previous study (Table 4) and it is apparent that, with the single exception of the youngest hooded rats which are as efficient as the oldest growing CFY rats, all the groups of CFY rats are twice as efficient as the hooded rats. Of course, such an expression of efficiency is only meaningful when the energy cost of protein turnover is being considered since the recycling of amino acids means that protein turnover *per se* should not necessarily affect amino acid requirements. As long as the dietary amino acid supply is sufficient for a particular growth rate, such a growth rate can be achieved at a low efficiency if the rate of protein synthesis is fast enough. This was made evident by the fact that the growth rate of the youngest hooded rats, 6·27%/d was similar to that of the youngest CFY rats (6·31%/d) even though the efficiency of synthesis was much lower in the hooded rats (Table 4). However, only the youngest hooded rats were able to maintain this high rate of protein synthesis so that, as they grew, the fall in the rate of protein synthesis together with the low efficiency resulted in a marked fall in the growth rate.

While the differences in efficiency of protein synthesis, as defined previously, are obvious between the two strains (see Table 4) it is difficult to draw unequivocal conclusions about strain differences in protein synthesis or degradation. Comparisons made on an age basis would indicate in general, similar or lower rates of synthesis and lower rates of degradation in the fast-growing rats. Comparisons made on a weight basis indicate a more complex pattern of differences with the fast-growing rats exhibiting higher rates of protein synthesis at the heavier weights and the lower rate of degradation at the lighter weights. For these reasons an emphasis on differences in efficiency of protein synthesis as defined here is less equivocal.

The effect of these differences in the efficiency of muscle protein synthesis and deposition on the energetic efficiency of growth in the whole animal depends on two factors, the energy cost of protein synthesis and the extent to which the efficiency of protein synthesis in muscle is also reflected in the whole animal (the last point is particularly important since protein synthesis in muscle accounts for only a small proportion of whole-body protein synthesis

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in the rat). We have not measured metabolic rates in rats in which we have measured whole-body protein turnover. However, assuming that in the hooded rats and CFY strain the metabolic rates are similar to those reported by other authors (e.g. Walker & Norton, 1970) and assuming that the tyrosine flux through the plasma is a reasonable index of whole-body protein turnover, we have reported that protein synthesis may account for about 20% of total heat production (e.g. Millward *et al.* 1976, Waterlow *et al.* 1978). Since protein synthesis in muscle accounts for less than 20% of whole-body protein synthesis (Waterlow *et al.* 1978, Millward *et al.* 1981) it is unlikely to account for more than a very small proportion of body heat production and will not be a major determinant of the over-all growth efficiency. Whether the apparent efficiency of protein synthesis in muscle is also reflected in the whole-body rate of protein synthesis and deposition cannot be determined without better information about the rates of whole-body protein synthesis in these animals.

Apart from a consideration of energy requirements the physiological significance of the efficiency of muscle protein synthesis in any other context is difficult to assess because it is not clear why proteins should be degraded at all after they have been synthesized in the cell. Because the rate of protein degradation appears to be a characteristic feature of individual tissues we might assume that some characteristic structural or functional feature of each tissue determines its degradation rate (see Millward, 1980*b*, Millward *et al.* 1981). Muscle appears to be unique since the rate of degradation appears to vary markedly throughout development, being faster in younger animals in all species where measurements have been made (Millward, 1980*a*). The present results demonstrate this. Because of the apparent correlation between growth rates and protein degradation in muscle we suggested previously that increased degradation may be perhaps associated with such events as myofibrillar splitting and re-modelling of the architecture of the contractile apparatus during growth (Millward *et al.* 1975). Such a hypothesis is supported by the observations that when growth is induced during work-induced muscle hypertrophy, for example, protein degradation in muscle is increased (Laurent *et al.* 1978; Laurent & Millward, 1980), and when muscle growth is suppressed in growing animals the rate often falls (e.g. Millward *et al.* 1975, Millward, Garlick, Nnanyelugo *et al.* 1976). These original ideas about the nature of changes in protein degradation in muscle need to be revised however with more recent information. For example Maruyama *et al.* (1978) observed that in chick muscle the high rate of protein degradation observed in 1-week-old birds, which normally falls with age, was maintained when growth was inhibited by amino acid deficiency. Furthermore, these results showed that in pectoral muscle of the chick while the degradation rate fell from 26·5%/d at 1 week to 10%/d at 2 weeks the fractional growth rate actually increased at this time. This means that in the chick at least the high rate of degradation in neonatal muscle can be dissociated from the growth process and may reflect some, as yet unknown, feature of postnatal development. Also, hormonal changes can alter the degradation rate. Thus, in the rat, increases in the growth rate of muscle following treatment with trembolone acetate (Vernon & Buttery, 1976) appear to result from a decrease in the degradation rate, a change which we have recently confirmed (J. G. Brown and D. J. Millward, unpublished observation). Thyroid hormones also appear to alter the growth rate of muscle by changing the degradation rate as well as the rate of protein synthesis (Brown & Millward, 1980, 1981). Findings such as these suggest that the degradation rate in muscle may change as a result of several influences including growth-induced changes, developmental changes in muscle structure and function and hormonal status. In the present results, the increased growth of muscle, compared with the rate which we have observed in our previous studies, appears to involve less degradation per unit of synthesis, suggesting that lower rates of degradation participate in the increased growth. Whether this reflects differences in the mechanism of myofibril proliferation, differences in the developmental changes in muscle structure and

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function or differences in the hormonal regulation of muscle growth in this strain, remains to be determined.

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