Myofibrillar protein turnover

Synthesis rates of myofibrillar and sarcoplasmic protein fractions in different muscles and the changes observed during postnatal development and in response to feeding and starvation

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Measurement of rates of synthesis of skeletal-muscle proteins in adult rats shows that the faster overall rate of turnover in diaphragm and soleus muscles compared with several other, more glycolytic, muscles is also exhibited by the myofibrillar proteins, since the ratio of sarcoplasmic to myofibrillar protein synthesis is similar for all muscles. Further, throughout postnatal development, when the overall turnover rate falls with age, parallel changes occur for the myofibrillar proteins, as indicated by a constant ratio of sarcoplasmic to myofibrillar protein synthesis (2.06) in the steady state after overnight starvation. Only in the youngest (4 weeks old) rats is a slightly lower ratio observed (1.72). These results indicate that, when changes in the overall turnover rate of muscle proteins occur, the relative turnover of the two major protein fractions stays constant. However, measurements in the non-steady state during growth and after starvation for 4 days show that the relative synthesis rates of the two fractions change as a result of a disproportionate increase in myofibrillar protein synthesis during growth and decrease during starvation. Thus the synthesis rate of the slower-turning-over myofibrillar protein fraction is more sensitive to nutritional state than is that of the sarcoplasmic protein. It is suggested that such responses may help to maintain constant tissue composition during non-steady-state conditions of growth and atrophy.

In skeletal muscle the major part of the intracellular protein mass consists of the myofibrils, the integrity of which is essential for the generation and transmission of the contractile force. The individual proteins of the myofibrils are nevertheless in a dynamic state, being continuously degraded and replaced at rates which vary according to the muscle fibre type (see Millward, 1980*a*) and age of the animal (see Millward *et al.*, 1975; Millward, 1980*b*; Bates & Millward, 1981). In addition to this motor function, muscle protein serves as an important source of gluconeogenic carbon in starvation, as well as a potential source of essential amino acids for protein synthesis in other tissues [see Young (1970) and Waterlow *et al.* (1978)]. To achieve this latter role, the overall rate of protein synthesis and degradation must be regulated, and this has been repeatedly demonstrated in a wide range of animal studies [see Waterlow *et al.* (1978) and Millward *et al.* (1981)] as well as in man (Rennie *et al.*, 1982). What is much less well documented is the relationship between the overall rates of muscle protein

synthesis and degradation and the turnover of the individual myofibrillar proteins, even though these were amongst the first to be studied with isotopic tracers (Schapira *et al.*, 1956; Velick, 1956). This is an important problem, not only because an understanding of the assembly and replacement of the contractile apparatus may help to explain some disease states in muscle, but also because the release of 3-methylhistidine (*N*^ε-methylhistidine) after the degradation of actin and some species of myosin heavy chain is widely used as a method for studying muscle protein degradation (Young & Munro, 1978). We have reported that the turnover of protein-bound 3-methylhistidine in skeletal muscle is slower than the overall turnover rate to the extent that it is not possible to account for urinary 3-methylhistidine excretion without postulating substantial non-muscle sources (Millward *et al.*, 1980). In the present study we examine the quantitative and qualitative relationship between the overall turnover rate of muscle protein and that of the myofibrillar proteins. The relative synthesis rates of the two

major protein fractions, the sarcoplasmic and myofibrillar proteins, is examined in a number of situations when the overall rate of protein synthesis is known to vary. Preliminary accounts of some of these studies have been presented previously (Bates & Millward, 1978; Waterlow *et al.*, 1978; Millward & Waterlow, 1978).

Experimental

Animals

All animals were male hooded Lister rats (Olac, Bicester, Oxon, U.K.) at various ages and weights (given as means $\pm$ s.d.) specified in the text. All were fed on stock Oxoid diet (Oxoid, Basingstoke, Hants, U.K.) *ad libitum*. The starved rats had their food removed at 10:00h and the experiments were performed 24 h or 4 days later.

Separation of sarcoplasmic and myofibrillar fractions

The two fractions were separated on the basis of their differential salt solubilities by a method similar to that of Helander (1961). All operations were done at 4°C or below. Approx. 2g of muscle was homogenized with a Polytron homogenizer in about 20ml of low-salt buffer (0.025 M-KH₂PO₄/0.025 M-K₂HPO₄, adjusted to pH 7.0 with KOH). Triton X-100 was added to 1% final concn., and the homogenate was left at 4°C for 1 h. After centrifugation at 2800g for 20 min, the supernatant was removed and an equal volume of 10% (w/v) trichloroacetic acid added to this. The resulting precipitate was washed twice with 10% trichloroacetic acid to remove free amino acids, and the protein pellet was the sarcoplasmic protein fraction, which was subsequently hydrolysed and analysed for tyrosine specific radioactivity. The precipitate from the low-salt buffer was washed twice more in low-salt buffer. Further washes appeared to be free of protein, as judged by the lack of precipitate after treatment with 10% trichloroacetic acid. The myofibrillar pellet was dissolved in 5 ml of high-salt buffer (0.3 M-KCl/0.1 M-KH₂PO₄/0.05 M-K₂HPO₄/4 mM-potassium pyrophosphate, pH 7.2). This was left overnight at 4°C, to ensure complete dissolution, and then centrifuged at 31 000g for 20 min. The supernatant was poured off, and 10 vol. of ice-cold water was added to re-precipitate the myofibrillar proteins. After standing for 2 h at 4°C, the myofibrillar proteins were centrifuged at 2800g for 20 min, and then redissolved by adding solid KCl (final concn. 0.5 M). This was again left to dissolve overnight at 4°C, centrifuged at 31 000g for 20 min and re-precipitated by adding 10 vol of ice-cold water. The resulting precipitate was the myofibrillar protein fraction, which was hydrolysed and analysed for tyrosine specific radioactivity.

In these studies the two protein fractions examined (sarcoplasmic and myofibrillar) represent most, if not all, of the non-collagen protein in muscle, each fraction therefore containing many proteins. No attempt was made to characterize the proteins present in the sarcoplasmic fraction. However, in the course of experiments in which aldolase was isolated from the sarcoplasmic fraction (Bates *et al.*, 1983), samples were run on sodium dodecyl sulphate/polyacrylamide gels and, as far as could be ascertained, no contractile proteins were present. Only when muscle is homogenized in a relaxing buffer (containing EGTA and pyrophosphate) and centrifuged at low speeds are myofibrillar proteins left in the supernatant (Etlinger *et al.*, 1975). As far as the myofibrillar fraction is concerned, the preparation procedure reported here results in a fraction which, according to our examinations on sodium dodecyl sulphate/polyacrylamide gels, contained the principal contractile proteins and relatively small amounts of soluble proteins. However, since rigorous characterization of the two fractions was not performed in each experiment, we can only assume that the two fractions were always of the same composition.

Protein-synthesis measurements

Rates of protein synthesis were determined by the constant-infusion method with [¹⁴C]tyrosine as described by Garlick & Marshall (1972) and Garlick *et al.* (1973), except that infusions were into a tail vein. In the experiments where the relative synthesis rates of protein fractions were measured, the labelling of [¹⁴C]tyrosine isolated from the protein fractions was determined in the same way as in the constant-infusion experiments.

Expt. (a): relative synthesis rates of sarcoplasmic and myofibrillar proteins in different muscles. Six adult (non-growing) rats weighing 433 ± 30 g were infused with L-[U-¹⁴C]tyrosine (10 Ci/mol; 4 μ Ci/ml; 0.5 ml/h). After 6 h they were killed by decapitation and several individual muscles were dissected for analysis. The muscles were diaphragm, combined gastrocnemius and quadriceps, soleus, plantaris, psoas and quadratus lumborum. Each muscle was minced with scissors on an ice-cold glass plate and divided into two. One half was used for measurement of total protein-synthesis rate and the other half was fractionated into sarcoplasmic and myofibrillar proteins to measure relative synthesis rates.

Expt. (b): relative synthesis rates of sarcoplasmic and myofibrillar proteins during growth. Groups of 12 rats at 35, 90, 300 and 400 g were used. At each weight six rats were fed and six rats were starved for 24 h, making eight groups in all. Each rat was injected intraperitoneally with 5 μ Ci of L-[U-¹⁴C]-tyrosine (450 Ci/mol; 25 μ Ci/ml of 0.9% NaCl). After 1 h the rats were killed by decapitation, and the

gastrocnemius and quadriceps muscles were removed and frozen in liquid N₂ before subsequent analysis as described above.

Expt. (c): effects of starvation on relative synthesis rates of sarcoplasmic and myofibrillar proteins. Eight rats weighing 405 ± 24 g were divided into two groups of four. One group was deprived of food for 4 days, after which time they weighed 339 ± 21 g. They were infused, together with the remainder, which had been well fed, with L-[U-¹⁴C]tyrosine (10 Ci/mol; $2 \mu\text{Ci/ml}$; 0.5 ml/h), and at the end of 6 h they were killed by decapitation. The gastrocnemius and quadriceps from one leg were used to measure total protein-synthesis rate, and those from the other leg to measure synthesis rates of sarcoplasmic and myofibrillar fractions.

Results and discussion

Turnover of myofibrillar proteins in different muscles

In the rat, as in most mammalian species, individual muscles comprise a mixed population of

fibre types, but muscles can be selected with a predominance of one fibre type (Ariano *et al.*, 1973). Thus diaphragm and soleus contain mainly slow oxidative fibres, and plantaris and quadratus lumborum contain mainly fast glycolytic fibres. The diaphragm and the soleus had significantly higher protein-synthesis rates than did the other muscles (Table 1a), which is consistent with previous published results (Goldberg, 1967; Short, 1969; Frayn & Maycock, 1979).

These differences in overall rates of protein synthesis did not result from differences in the pattern of protein synthesis, however, since there were no significant differences in the ratios of the synthesis rates of the sarcoplasmic and myofibrillar proteins in the different muscles. In each case the sarcoplasmic proteins were synthesized (and therefore degraded, since those rats were not growing) at twice the rate of the myofibrillar proteins. Thus, as described by Lebherz *et al.* (1978), who examined the relative synthesis rates of proteins from breast and leg muscle, in adult fowl and young chicks, and by Laurent *et al.* (1978), who examined anterior and

Table 1. *Synthesis rates of sarcoplasmic and myofibrillar proteins in various skeletal muscles in fed adult rats (a), in muscle from fed and overnight-starved rat throughout development (b) and in muscle from fed and 4-day-starved adult rats (c)*

In (a) the rates of synthesis of mixed protein and the sarcoplasmic and myofibrillar protein fractions were measured by the constant infusion of [¹⁴C]tyrosine over 6 h. In (b) the synthesis rates were determined as the relative labelling of [¹⁴C]tyrosine isolated from sarcoplasmic and myofibrillar proteins of combined gastrocnemius and quadriceps muscles (G + Q) from rats killed 60 min after the injection of trace amounts of [¹⁴C]tyrosine. In (c) measurements were made on combined gastrocnemius and quadriceps muscles from rats infused with [¹⁴C]tyrosine for 6 h. The values are means $\pm$ S.D. for four rats (a and c) or six rats (b).

	Body wt. (g)	Nutritional state	Muscle	Protein synthesis	
				Rate (%/day)	Sarcoplasmic myofibrillar (ratio)
(a)	433 ± 30	Fed	Diaphragm	8.31 ± 1.98	2.11 ± 0.30
			Soleus	8.92 ± 1.08	2.04 ± 0.34
			G + Q	4.46 ± 1.15	1.99 ± 0.12
			Plantaris	4.78 ± 1.35	2.04 ± 0.12
			Psoas	4.46 ± 0.98	2.15 ± 0.22
			Quadratus lumborum	3.95 ± 0.71	2.10 ± 0.31
(b)	35.6 ± 1.9	Fed	G + Q		1.30 ± 0.05
	28.7 ± 3.2	Starved	G + Q		1.72 ± 0.16
	95.6 ± 10.2	Fed	G + Q		1.39 ± 0.15
	87.3 ± 2.5	Starved	G + Q		2.08 ± 0.11
	309 ± 8	Fed	G + Q		1.54 ± 0.08
	268 ± 14	Starved	G + Q		2.03 ± 0.19
	450 ± 30	Fed	G + Q		1.75 ± 0.10
	473 ± 21	Starved	G + Q		2.08 ± 0.15
(c)	405 ± 24	Fed	G + Q		
			Sarcoplasmic	6.12 ± 0.75	1.76 ± 0.01
			Myofibrillar	3.48 ± 0.40	
		Starved 4 days	G + Q		
			Sarcoplasmic	4.29 ± 0.60	2.30 ± 0.09
			Myofibrillar	1.87 ± 0.30	

posterior latissimus dorsi muscles, differences in the overall synthesis rates between muscles are also observed when measurements are made on the myofibrillar proteins. In the adult fowl, however, in contrast with the present results in the rat, there was no difference between turnover rates of myofibrillar and sarcoplasmic proteins. Loble & Lovie (1979) also reported similar ratios of labelling of tyrosine from the two protein fractions in the rabbit, results which we have confirmed (P. C. Bates, G. J. Laurent & D. J. Millward, unpublished work). In the pig, Perry (1974) reported faster decay rates of sarcoplasmic proteins labelled with $\text{Na}_2^{14}\text{CO}_3$, although the ratio of sarcoplasmic to myofibrillar rates was only 1.2. In man, however, Halliday & McKeran (1975) reported the ratio of sarcoplasmic to myofibrillar synthesis rates as 2.7. Since there is no reason to believe that any of these reports involved technical difficulties, it would appear that there are species differences in the relative turnover rates of the two protein fractions in skeletal muscle.

Developmental changes in the relative turnover rates of sarcoplasmic and myofibrillar proteins

During postnatal development there are marked changes in the turnover rate of muscle proteins, the rate falling from a high rate at birth to the lower muscle-specific rate at some time after attainment of sexual maturity (Millward *et al.*, 1975; Millward, 1980b).

Measurements were made of the relative turnover rate of the two protein fractions throughout postnatal development to see whether there were selective changes in the turnover of the two fractions. In this case the ratio of synthesis rates was assumed to be equal to the ratio of specific radioactivities of tyrosine isolated from the two protein fractions 60 min after a single injection of trace amounts of the ^{14}C -labelled amino acid. This assumption is justified if the precursor labelling is greater than that of the different proteins throughout the incorporation period. In one experiment, groups of both fed and starved rats were injected with [^{14}C]tyrosine and measurements were made of the free and protein-bound tyrosine after 1 h. In each group the free tyrosine labelling was more than 80 times that of the protein-bound tyrosine. Thus an incorporation period of 1 h is justified.

The relative synthesis rates of the two protein fractions throughout development are shown in Table 1(b). For the overnight starved rats, in the youngest animals the ratio of sarcoplasmic to myofibrillar protein-synthesis rates was 1.72 (± 0.16), whereas in the three older groups the ratio was higher at 2.06 (± 0.15).

We believe that these values can be considered to be equal to the rates of turnover (i.e. the steady-state replacement rates) of the two protein fractions.

Because starvation for 24 h is long enough to eliminate growth, but not long enough to initiate losses of muscle protein (Millward & Waterlow, 1978; Garlick, 1980), there is a steady state in which protein-synthesis rates are equal to degradation rates. Possibly in the youngest rats losses of muscle protein had begun to occur with synthesis rates below rates of degradation, since younger rats are more susceptible to starvation (see Garlick, 1980). Since more prolonged starvation increased the ratio of synthesis rates of the two fractions (Table 1c), the actual steady-state ratio of synthesis rates in the youngest group is likely to be, if different, greater rather than less than the measured value. Thus we would not expect the extent of the non-steady state in this group to be sufficient to invalidate our conclusion that the faster overall rate of muscle protein turnover in young rats (Millward *et al.*, 1975; Millward, 1980b) involves both myofibrillar and sarcoplasmic proteins. The increase in the ratio from 1.72 at 35 g to 2.08 at 100 g body wt. implies that the myofibrillar proteins exhibit a developmental fall in turnover which is more extensive than the overall rate between 35 and 100 g. The constant ratio between 100 and 400 g body wt. implies that the turnover of the myofibrillar proteins falls in parallel with the overall rate in older rats. According to previous measurements in this rat strain, degradation rates of mixed muscle proteins at weaning were 22.7%/day, falling to 4.5%/day at maturity (Millward *et al.*, 1975). Since the ratio of sarcoplasmic to myofibrillar proteins is 1:2.4 according to our measurements, ratios of 1.72 at weaning and 2.08 at maturity for relative turnover rates of sarcoplasmic to myofibrillar proteins indicate that myofibrillar protein turnover rates fall from 18.7 to only 3.42%/day, equivalent to half-lives of 3.7 and 20.3 days in the gastrocnemius and quadriceps muscles.

Why these developmental changes occur is not known. There are developmental changes in the contraction characteristics of individual muscles (see, e.g., Gutmann *et al.*, 1974), although the differences are less marked than those which exist between individual adult muscles. The gastrocnemius and quadriceps muscles used in the present studies contain mixed populations of fibres. Although our measurements of synthesis rates of the red and white parts of the gastrocnemius have never revealed differences as marked as those that we observe between soleus and plantaris muscle (D. J. Millward, unpublished work), nevertheless changes in fibre composition with age could be part of the reason for the change in the overall rate of protein turnover. However, since the developmental fall in turnover is observed in all muscle types (see Millward, 1980b), a change in fibre type with age is unlikely to be the only reason for the developmental fall in turnover in gastro-

Table 2. Relationship of skeletal-muscle growth rate and the ratio of synthesis rates of the sarcoplasmic and myofibrillar fractions

Growth rates for the first four groups are those observed previously in rats of this strain at each weight studied in the present experiments, as described in Table 1(b). The fifth (zero growth) group is the average for the 24h-starved groups, with the exception of the youngest group shown in Table 1(b), where a steady state cannot definitely be assumed to occur. The sixth group is the 4-day-starved group described in Table 1(c).

Total muscle protein growth rate (%/day)	Relative synthesis rates of sarcoplasmic and myofibrillar proteins
6.3	1.30
2.3	1.39
0.7	1.54
0.3	1.75
0.0	2.06
-6.8	2.30

cnemius and quadriceps. The presence of mixed fibre types, together with their large size and ease of dissection, makes them more likely to be representative of the entire skeletal-muscle mass.

Effects of feeding and starvation on relative synthesis of rates of muscle proteins

At each age, the relative synthesis rates of the sarcoplasmic and myofibrillar proteins were measured in the fed state (at 09:30h in rats fed *ad lib.*) as well as after overnight starvation. The results are shown in Table 1(b). The ratio of synthesis rates was lower in fed than in starved animals at each age. In addition, the ratio was lower in the youngest fed group and increased progressively with age. A further experiment was performed to examine whether more prolonged starvation further increased the ratio of synthesis rates. In this case the rats were infused with [14 C]tyrosine for 6h so that actual as well as relative synthesis rates could be determined. The overall synthesis rates fell by over 40%, but, as shown in Table 1(c), there was a disproportionate fall in the synthesis rate of the myofibrillar proteins, so that the ratio of synthesis rates of the two fractions increased from 1.76 ± 0.01 in the fed state to 2.30 ± 0.09 in the starved state.

The results show clearly that the pattern of protein synthesis changes in muscle according to the nutritional state and the age. Furthermore, it would appear that the synthesis rate of the myofibrillar proteins changes to a disproportionate extent relative to the synthesis rate of the sarcoplasmic proteins. Although all the fed animals were examined at the same time of the day and were in one sense in the same nutritional state, younger rats have a

higher food intake, exhibit a faster fractional growth rate, and in this strain exhibit a higher RNA activity (the rate of protein synthesis per unit RNA; Millward *et al.*, 1975). If this is interpreted as indicating that in younger fed rats muscle protein synthesis is more 'activated' than in older fed rats, then, with the progressive shut-down of protein synthesis from the youngest fed, through oldest fed, 24h-starved to 4-days-starved rats, there is a disproportionate decrease in the synthesis rate of the myofibrillar proteins.

It is important to consider the possibility that these findings of differential changes in the synthesis rates of the two fractions are an artifact resulting from the release of myofilaments described by Etlinger *et al.* (1975) into the sarcoplasmic fraction. Differential release of these filaments in the fed and starved states could have been responsible for the changes in the relative synthesis rates on starvation. We do not think that this occurred, for the following reasons. Firstly, the preparation procedure that we used is unlikely to release myofilaments, since a non-relaxing buffer was used (i.e. there was no EGTA or pyrophosphate). Also, the extraction lasted for 1h, in contrast with the shorter time necessary for myofilament release, and the homogenate was centrifuged at a higher speed than that which would allow myofilaments to remain un-pelleted. Secondly, and more importantly, we have not observed differences in the relative labelling of the two fractions with time, which would be expected (according to the findings of Etlinger *et al.*, 1975) if myofilament release occurred. Thus in preliminary experiments, when individual young fed rats were killed at 10, 20, 40 and 60min after injection, the ratio of labelling of the sarcoplasmic to myofibrillar fractions did not change with time (i.e. 1.29 ± 0.03). In older fed rats, measurements at 1h (Table 1b) gave values identical with those obtained after a 6h constant infusion (Table 1c). In contrast, Etlinger *et al.* (1975) reported that the relative labelling of the released filaments and the residual myofibrillar proteins changed remarkably between 30min and 3h. Thirdly, the assumption that the present results of differential changes in synthesis rates of the two protein fractions are real and not an artifact caused by differential release of highly labelled myofilaments is supported by the further experiments described in the following paper (Bates *et al.*, 1983).

There have been few previous studies of the relative turnover rates of muscle proteins in different nutritional states. Low & Cerauskis (1977) reported measurements of relative labelling of rat muscle proteins 8h after pulse labelling which indicated no change on starvation. However, the relative labelling after 8h would only be a valid indication of relative synthesis rates if the amino acid precursor was still more highly labelled than the

protein product, and this was not indicated in their study.

The observation that relative synthesis rates of the two protein fractions change according to the nutritional state can to some extent be explained on the basis that it is a necessary part of a regulatory mechanism involved in the maintenance of a constant tissue composition of proteins (and protein fractions) with different turnover rates. Thus during tissue growth the maintenance of a constant composition necessitates the same absolute increase in synthesis for all proteins. This would mean that the increase in the synthesis rate for each protein will be an increasing proportion of the overall rate for the slower-turning-over proteins, which is what is observed here for the myofibrillar proteins. Furthermore, the possibility that the changes in relative synthesis rates are part of a regulatory mechanism is supported by the observations that the extent of the change varies with the growth rate, as shown in Table 2. Included in Table 2 is the ratio of synthesis rates in the 4-day-starved rats, in which the growth rate was negative. Clearly in this case it is not possible to achieve the required net change (i.e. -6.8%/day) by suppressing synthesis alone, since the net degradation rate is greater than the steady-state degradation rate (4.3%/day, equal to the weighted mean of the synthesis rates of the two fractions shown in Table 1c). Thus an increase in degradation must also occur. Nevertheless a disproportionate suppression of the synthesis rate of the slower-turning-over proteins will help to achieve a constant ratio of amounts of the two fractions removed. What is not indicated in these studies is the extent to which these changes in relative synthesis rates involve the synthesis of the two protein fractions as a whole, or whether individual proteins in each fraction also undergo differential changes in synthesis rates of feeding and starvation. This is examined in the following paper (Bates *et al.*, 1983).

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