Skeletal-Muscle Growth and Protein Turnover

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Because of turnover, protein synthesis and breakdown can each be involved in the regulation of the growth of tissue protein. To investigate the regulation of skeletal-muscle-protein growth we measured rates of protein synthesis and breakdown in growing rats during development on a good diet, during development on a marginally low-protein diet and during rehabilitation on a good diet after a period of severe protein deficiency. Rates of protein synthesis were measured *in vivo* with a constant intravenous infusion of [^{14}C]-tyrosine. The growth rate of muscle protein was measured and the rate of breakdown calculated as breakdown rate = synthesis rate – growth rate. These measurements showed that during development on a good diet there was a fall with age in the rate of protein synthesis resulting from a fall in capacity (RNA concentration) and activity (synthesis rate per unit of RNA). There was a fall with age in the breakdown rate so that the rate was highest in the weanling rats, with a half-life of 3 days. There was a direct correlation between the fractional growth and breakdown rates. During rehabilitation on the good diet, rapid growth was also accompanied by high rates of protein breakdown. During growth on the inadequate diet protein synthesis rates were less than in controls, but growth occurred because of decreased rates of protein breakdown. This compensation was not complete, however, since ultimate muscle size was only one-half that of controls. It is suggested that increased rates of protein breakdown are a necessary accompaniment to muscle growth and may result from the way in which myofibrils proliferate.

Since the proteins of skeletal muscle, including those of the myofibril, exhibit considerable turnover (Millward, 1970a; Waterlow & Stephen, 1968), regulation of the growth of muscle protein could be exerted through changes in either protein synthesis or breakdown. In muscle, protein synthesis is almost certainly a primary site of regulation since its rate is very sensitive to dietary changes (Millward, 1970b; Waterlow & Stephen, 1968; Millward & Garlick, 1972). The central question considered here is the importance of protein breakdown as a regulatory process in muscle and in particular whether suppression of breakdown can occur to enhance growth.

It appears likely that the overall control of the liver protein mass may be exerted through changes in the rate of proteolysis (Garlick *et al.*, 1974), since insulin (Mortimore & Mondon, 1970), amino acids (Woodside & Mortimore, 1972) and energy supply (Parrilla & Goodman, 1974) all suppress protein breakdown in this tissue. In muscle, regulatory changes in the rate of protein breakdown have been described in several studies (Goldberg, 1969a,b; Millward, 1970b; Young *et al.*, 1971).

These results suggest that in skeletal muscle, as in the liver, changes in the rate of breakdown are an important component in the regulation of muscle

protein mass. However, in all these experiments the conclusions were based on the rate of loss of radioactive isotope from skeletal muscle *in vivo*. Although in each of these studies particular care was taken to avoid errors arising from reutilization of isotope (Millward, 1970a) the results are not entirely unequivocal. Uncertainty about the kinetics of muscle protein turnover, particularly that of myofibrillar proteins, could seriously complicate the interpretation of decay experiments. Some authors have discussed and discounted this possibility (Goldberg, 1969b), but other studies suggest that the turnover of myofibrillar proteins might be non-random (Morkin, 1970), perhaps exhibiting lifetime kinetics, as Dreyfus and co-workers suggested several years ago (Dreyfus *et al.*, 1960).

We have therefore attempted to assess the way in which protein synthesis and breakdown change during muscle growth under several different conditions: in well-fed and marginally malnourished rats and in rats recovering on a good diet from a period of severe protein deficiency. The rate of protein synthesis was determined *in vivo* by constant infusion of a labelled amino acid (Waterlow & Stephen, 1968; Garlick *et al.*, 1974). The results obtained by this method do not depend on any assumptions about the

kinetics of turnover, i.e. whether it is random or 'lifetime'. We have also measured growth rate of the muscle protein mass and from these two measurements can estimate the rate of breakdown, since net gain of protein = protein synthesized - protein broken down.

Some of the results have been presented in a preliminary form (Millward *et al.*, 1974b, 1975a).

Experimental

Animals and diets

Rats of two strains were studied. In experiment (I) we compared well-fed and marginally malnourished rats from colonies of a hooded strain bred in this department for many generations. One colony had been fed on a cubed diet adequate for all stages of development (net dietary protein energy 10%). The other colony had been fed on a cubed diet adequate for maintenance in adults but inadequate for maximum early growth (net dietary protein energy 6.8%). The composition of the diets and the characteristics of the colony are described elsewhere (Payne & Stewart, 1972; Stewart *et al.*, 1975). In the colony reared on the diet of lower protein content the birth weight is low and the perinatal mortality high; the rate of growth in early life is low and the ultimate adult size decreased to about one-half of that of well-fed rats. We refer to these rats as marginally malnourished.

In both groups measurements of the amount of muscle protein and of its turnover rate were made at different ages from 23 to 330 days.

In experiment (II) Wistar rats purchased commercially were made severely malnourished by feeding them on a purified protein-free diet (Millward *et al.*, 1974a). The rats were started on this diet at the age of about 50 days and maintained on it for 30 days. They were rehabilitated by feeding them on a standard commercial pelleted diet. Protein turnover rate was measured at intervals after the beginning of rehabilitation.

Control rats were fed on the commercial diet throughout. One group of controls (weight controls) was killed at a body weight corresponding to that of the experimental rats during the second week of rehabilitation. Another group (age controls) was killed when their age was that of the experimental rats at the beginning of rehabilitation.

Muscle composition and rate of growth

When each rat was killed the gastrocnemius and quadriceps muscles were dissected out and weighed. Total non-collagen protein, DNA and RNA were measured as described by Millward *et al.* (1974a). The rate of increase in muscle protein (mg/day) was

calculated for the period before and after death, and the mean taken. This mean, which is an estimate of the absolute growth rate at the time of death, was divided by the protein content at this time to obtain a fractional rate of growth (percentage growth/day), which could be compared directly with the fractional rate of synthesis.

In experiment (I) to calculate the growth rates in the weanling rats, it was assumed that the protein mass of the gastrocnemius and quadriceps muscles formed the same proportion of body weight at birth as at weaning. This gave values of 5 and 4 mg respectively for the protein content of the combined muscles at birth in the well-fed and marginally malnourished rats. The estimate is approximate, but it will not result in a significant error in the calculation. For the oldest rats in each group the growth rate at death was assumed to be equal to the growth rate over the period preceding death.

In experiment (II) the growth rates of the rats before re-feeding (i.e. chronically malnourished) and of the two control groups are necessarily approximate, since they were estimated from the rate of change of body weight at the time of death. The increase in protein mass after 1 day of re-feeding was not significant, and so the growth rate was not calculated at this time.

The standard deviations of the growth rates were calculated from the variances of the mean muscle protein contents at the time of death and at the time-points immediately before and after this time (i.e. from the data used to calculate the growth rates). Because the growth rates for the two oldest groups in experiment (I) and the 14-day-re-fed group in experiment (II) were calculated, of necessity, only from the rate of increase before death, a comparable standard deviation could not be calculated.

Rate of muscle protein synthesis

The rate of protein synthesis was measured *in vivo* by the constant intravenous infusion method (Waterlow & Stephen, 1968; Garlick *et al.*, 1974). Rats were wrapped in a cloth so that the tail was exposed. A 24-gauge needle attached to a length of polypropylene tubing was inserted into a lateral tail vein. The needle was taped in position with zinc oxide tape and the cannula tubing connected to a 10 ml glass syringe on a slow-infusion apparatus. L-[U-¹⁴C]Tyrosine (12.9 μ Ci/ μ mol) in 0.9% NaCl (4 μ Ci/ml) was infused at a rate of 2 μ Ci/h for 6 h. At the end of the infusion the rats were removed from the cloth, the infusion was stopped and the rats were immediately killed by decapitation. Blood was collected from the neck, and the gastrocnemius and quadriceps muscles from each leg were removed and frozen in solid CO₂. One each of the muscles was analysed for protein and RNA content and the others

were analysed for the specific radioactivity of free and protein-bound tyrosine as described by Garlick & Marshall (1972). In the albino rats (experiment II) only the gastrocnemius was analysed.

The fractional synthesis rate was calculated from the ratio of the specific radioactivities of free (S_f) and protein-bound (S_B) tyrosine in the tissue by the equation:

$$\frac{S_B}{S_f} = \left[\frac{R}{(R-1)} \cdot \frac{(1-e^{-k_s t})}{1-e^{-Rk_s t}} \right] - \frac{1}{R-1}$$

where R = ratio of the pool sizes of protein-bound and free tyrosine in muscle, taken as 400 (Garlick *et al.*, 1974); k_s = fractional synthesis rate. The derivation of this equation has been discussed (Garlick *et al.*, 1974).

The fractional breakdown rate was calculated by subtracting the fractional growth rate from the fractional synthesis rate. The standard deviation of the fractional breakdown rate was obtained by vectorial addition of the standard deviations of the fractional synthesis and growth rates. The synthesis rate per unit of RNA was determined by dividing the fractional synthesis rate by the RNA/protein ratio to give the amount of protein synthesized/day per g of RNA. We have described this quantity as the efficiency of protein synthesis (Millward *et al.*, 1973); perhaps a better term would be activity of RNA.

Results

Growth and muscle protein turnover on a normal diet

The results in this and the following section were obtained in rats of the hooded strain (experiment I). The body weights at different ages are shown in Fig. 1. The changes in muscle composition are shown in Table 1.

In terms of cellular growth, muscle growth for this colony was typical for the rat. Thus during the experimental period the 22-fold increase in muscle protein resulted from both hypertrophy and hyperplasia. There was an almost sixfold increase in cell size, or more correctly DNA-unit size (Cheek *et al.*, 1971), as well as a fourfold increase in DNA or cell number. These measurements would have included, of course, various cell types (connective tissue cells, adipose, etc.), but it has been assumed that constituents of non-contractile cells account for no more than a few per cent of the total (Cheek *et al.*, 1971; Young, 1974). The growth rate of muscle protein fell with age (Table 1 and Fig. 1). The highest rate, in the weanling rats, was 6.2%/day; if this rate were maintained, muscle protein would double every 11 days.

In the rat, growth rates of muscle protein in excess of this have seldom been recorded, even in individual muscles over short periods. For example, the fractional growth rate calculated from published reports

of muscle growth during work-induced hypertrophy of the plantaris muscle (Goldberg, 1967), during the transient hypertrophy of the diaphragm after unilateral phrenectomy (Turner & Garlick, 1974), and during catch-up growth of rehabilitated rats (Table 3) is less than 7%/day in each case. [The muscle growth rate reported by Young and co-workers of 12%/day during catch-up growth is quite exceptional (Young *et al.*, 1971).]

In the youngest rats the rate of protein synthesis was 28.6%/day, falling to 4.9%/day in adult rats 330 days old (Fig. 2). This fall resulted in part from a fall in RNA concentration, and in part from a decrease in the activity of the RNA [g of protein synthesis/day per g of RNA (Millward *et al.*, 1973) (Table 2)]. In the weanling rat the activity (19.2 g of protein/day per g of RNA) was almost twice that found in the adult (11.5 g of protein/day per g of RNA). The results in Fig. 2 show that the breakdown rate, calculated as the difference between synthesis and growth, was 22.4%/day at weaning, equivalent to a half-life of 3.1 days. At 330 days the breakdown rate had fallen to one-fifth of this value. It appears therefore that in muscle high breakdown rates accompany rapid growth; in

Fig. 1. Growth curves of rats fed on a normal diet or on a diet marginally low in protein

●, Normal net dietary protein energy = 10%; ○, malnourished net dietary protein energy = 6.8% for eleven generations. Each point is the mean (± 1 s.d.) body weight of four to eight male littermates.

Table 1. *Growth of muscle protein and muscle composition throughout development in well-fed and marginally malnourished rats (experiment I)*

Measurements were made on the gastrocnemius and quadriceps muscle of male littermates (four to eight rats) of a hooded strain. The fractional growth rate of the muscle protein mass was calculated from the mean of the absolute growth rates (in mg of protein/day) for the period before and after death, by dividing by the mass of protein at death. In the case of weanling rats it was assumed that at birth the muscle protein mass was 5 and 4 mg respectively and for the 330-day-old rats the growth rate is that of the period preceding death. The results are expressed as the mean $\pm$ 1 s.d. The s.d. of the growth rates was calculated where possible from the variances of the mean protein content of the muscles.

	Age (days)	Weight (g)	RNA DNA	Protein DNA	Muscle protein (mg)	Fractional growth rate (%/day)
Well-fed	23	37 $\pm$ 1	2.1 $\pm$ 0.1	141 $\pm$ 16	49 $\pm$ 4	6.27 $\pm$ 0.25
	46	70 $\pm$ 5	1.9 $\pm$ 0.1	167 $\pm$ 11	145 $\pm$ 7	2.97 $\pm$ 0.21
	65	116 $\pm$ 8	2.1 $\pm$ 0.2	255 $\pm$ 24	230 $\pm$ 31	1.71 $\pm$ 0.07
	130	233 $\pm$ 32	2.1 $\pm$ 0.1	402 $\pm$ 14	453 $\pm$ 30	0.72 $\pm$ 0.04
	330	511 $\pm$ 84	3.1 $\pm$ 0.52	740 $\pm$ 146	1074 $\pm$ 139	0.29
Marginally malnourished	30	17 $\pm$ 3	1.0 $\pm$ 0.1	80 $\pm$ 4	20 $\pm$ 4	5.64 $\pm$ 0.58
	60	35 $\pm$ 4	2.9 $\pm$ 0.4	282 $\pm$ 43	73 $\pm$ 10	4.06 $\pm$ 0.54
	120	135 $\pm$ 43	2.1 $\pm$ 0.1	384 $\pm$ 16	325 $\pm$ 109	0.94 $\pm$ 0.12
	185	209 $\pm$ 31	2.1 $\pm$ 0.2	438 $\pm$ 59	470 $\pm$ 42	0.30 $\pm$ 0.07
	330	276 $\pm$ 51	2.7 $\pm$ 0.5	595 $\pm$ 36	570 $\pm$ 47	0.12

this group of well-fed rats there was a direct relationship between growth rate and breakdown rate (Fig. 3).

Growth and protein turnover during marginal malnutrition

The results are shown in the second part of Table 1, in Table 2 and in Figs. 1, 2 and 3. At 30 days, body weight and muscle weight were less than one-half of those in the well-fed rats. Between 60 and 120 days the rate of growth improved, so that the absolute gains in body weight, muscle weight and muscle protein were similar to the controls. Later the growth rate again declined, so that in adult malnourished rats at 330 days body weights and muscle weight were little more than one-half those of controls. The final deficit in muscle protein resulted from equal decreases in cell size and cell number. It seems that in spite of the low weight at weaning a considerable degree of catch-up growth was possible, but only up to the time when the rats became sexually mature. When the fractional growth rates of muscle protein are calculated, it is apparent that at 30 days the malnourished rats achieved a nearly normal growth rate, and that the age-dependent fall in fractional growth rate was not as pronounced as in the controls (Fig. 2).

In contrast with the near-normal growth rate, the rate of muscle protein synthesis at 30 days was less than one-half that of the well-fed rats and was still considerably decreased at 60 days. These low synthesis rates resulted from an apparent inability to achieve the high rates of synthesis/unit of RNA which were observed in the young well-fed rats. The RNA concentrations were not depressed. It follows

that if the fractional growth rates were nearly normal at a time when the synthesis rates were decreased, the breakdown rates must also have been low. At weaning the breakdown rate was less than one-half that of the controls, and in contrast with the well-fed rats, there was no obvious relationship with the growth rate since the breakdown rate did not markedly change throughout development (Fig. 3).

Growth in these malnourished rats appeared to be more efficient than in the well-fed animals, in that for a given amount of protein synthesis more net growth was achieved. Thus at weaning 41% of protein synthesis was used for growth, compared with only 22% in the controls.

Rehabilitation of previously malnourished rats

The animals in this experiment were albino rats that had been fed on a protein-free diet for 30 days. At the end of this time they were losing muscle protein, so that there was a negative fractional growth rate (Table 4). As we have reported (Waterlow & Stephen, 1968; Millward & Garlick, 1972; Millward *et al.*, 1973), on the protein-free diet the rate of synthesis was greatly decreased (2.7%/day; Fig. 4); the RNA concentration in muscle was extremely low (Table 3) and the activity of the RNA in protein synthesis was also decreased (Table 4). After 1 day of re-feeding the rats on average gained 8 g in body weight, but there was virtually no increase in muscle protein. This lag in the increase in muscle protein was observed also by Mendes & Waterlow (1958) who noted, in contrast, an immediate increase in liver protein when rats deprived of protein were given a good diet.

The synthesis rate doubled in the first day; this was achieved almost entirely by an increase in efficiency, since the RNA content increased more slowly.

After 3 days of feeding the adequate diet, growth rate of muscle protein was similar to that found in normal weanling rats in experiment (I). The synthesis rate had again increased, largely through an increase in RNA content to a value that was approaching that found in normal rats growing at similarly rapid rates. As Fig. 4 shows, these rats were able to achieve a rapid rate of muscle growth by maintaining, at least for a time, the low rate of breakdown characteristic of the period of protein deprivation.

After 8 days of re-feeding the picture had changed. The high rate of growth was maintained; the synthesis rate had again almost doubled, through an increase in both capacity and activity of RNA. By this stage the breakdown rates had also increased, so that the pattern was similar to that found in very young rapidly growing rats. Compared with the weight controls, during the second week of re-feeding, rates of growth, synthesis and breakdown were all increased, and there was an enhanced activity of tissue RNA.

Discussion

Limitations on the growth of muscle proteins

Because tissue proteins turn over, growth regulation can be achieved by alteration of the rate of either protein synthesis or breakdown, as long as turnover is rapid compared with growth. In the liver the breakdown rate seems to be the key control point, since the rapid alterations in protein content which occur in response to feeding and fasting (e.g. Millward *et al.*, 1974a) occur with no change in the fractional synthesis rate and therefore must reflect changes in the rate of proteolysis (Millward & Garlick, 1972; Garlick *et al.*, 1974; Millward *et al.*, 1975b). In contrast with this straightforward situation the present

Fig. 2. Growth and protein turnover rates and RNA/protein ratios in skeletal muscle of well-fed and marginally malnourished rats throughout development

The measurements of rates of protein synthesis, growth rates of muscle protein and of RNA/protein ratios are described in Table 1. The breakdown rate was calculated as the difference between the synthesis and growth rates. Each point is shown as the mean (± 1 s.d.) of four to eight male littermates. ●, Well-fed rats; ○, marginally malnourished rats.

Table 2. Fractional rate of protein synthesis (F.S.R.) and the activity of muscle RNA from the well-fed and marginally malnourished rats (experiment I)

Rates of protein synthesis were measured by the constant intravenous infusion of [¹⁴C]tyrosine. The activity of RNA was determined by dividing the F.S.R. by the RNA/protein ratio. The rats are as described in Table 1, and results are means ± 1 s.d.

Well-fed rats			Marginally malnourished rats		
Age (days)	F.S.R. (%/day)	Activity of RNA (g of protein/day per g of RNA)	Age (days)	F.S.R. (%/day)	Activity of RNA (g of protein/day per g of RNA)
23	28.6 $\pm$ 2.6	19.2 $\pm$ 1.7	30	13.8 $\pm$ 2.0	11.4 $\pm$ 1.9
46	16.1 $\pm$ 2.3	14.2 $\pm$ 1.1	60	10.9 $\pm$ 1.4	11.0 $\pm$ 1.2
65	11.5 $\pm$ 2.0	13.8 $\pm$ 3.0	120	6.5 $\pm$ 0.4	11.8 $\pm$ 0.6
130	5.3 $\pm$ 0.4	10.1 $\pm$ 1.2	185	6.3 $\pm$ 2.0	14.4 $\pm$ 5.3
330	4.9 $\pm$ 0.6	11.5 $\pm$ 1.0	330	4.2 $\pm$ 0.7	9.1 $\pm$ 0.5

results indicate that in muscle, growth regulation is more complex since alterations in the breakdown rate, far from facilitating growth, may well limit it. In the rats studied here the breakdown rate was directly proportional to the growth rate, so that rapid growth on a good diet was accompanied by high rates of protein breakdown, necessitating even higher rates of protein synthesis.

The extent to which rapid protein turnover would limit growth depends on the extent to which increased demands for amino acids and energy imposed by the elevated rates of protein synthesis can be met. Amino acid supply is not liable to be rate-limiting, since the demands for amino acids are largely met by protein breakdown in the tissue, little more than the needs for growth being required from exogenous sources. Only a small fraction of amino acids cannot be recycled because of post-translational modifications (e.g. methylated histidine and lysine). Energy supply, on the other hand, may well be limiting, since it must be supplied at the overall rate of protein synthesis. The energy cost of protein synthesis is substantial (Kielenowski, 1972; Payne & Waterlow, 1971) and in growing human subjects a shortage of energy may often limit growth especially during catch-up (Ashworth, 1969; Payne & Waterlow, 1971).

This relationship between growth and turnover can be modified, however, since in the weanling marginally malnourished rats growth rates were near normal even though breakdown rates were decreased. We could call this an adaptive response, but it was not complete, as catch-up did not occur. For this to have been achieved the breakdown rate in the older rats would have to have fallen below the observed 4–5%/day. That this did not occur suggests that this may be the lower limit to the breakdown rate compatible with normal cellular function.

The developmental fall in muscle protein turnover demonstrated here has been observed previously (Waterlow & Stephen, 1968; Srivastava & Chaudhary, 1969). To a certain extent these developmental

changes are inevitable because of the nature of rat muscle growth. In these animals muscle growth involves both cellular hypertrophy and hyperplasia, the hypertrophy involving a fivefold increase in cellular protein content (Table 1). However, it appears that the cellular RNA content cannot be expanded by more than a limited extent. In our experience a value of about 3 is the upper limit to the RNA/DNA ratio in skeletal muscle, although higher values have been occasionally reported (e.g. Young *et al.*, 1971). Because of this, cellular hypertrophy will result in a fall in the RNA/protein ratio and the fractional rate of protein synthesis will fall. In fact, there is an additional decline in the activity of RNA so that the developmental fall in synthesis rate is even greater than would otherwise obtain.

Fig. 3. Relationship between growth and breakdown rates of muscle protein throughout development in well-fed (●) and marginally malnourished (○) rats

The data are the same as those in Table 1 and Fig. 2. A linear-regression analysis of the data from well-fed rats indicates the relationship: breakdown rate = 3.07 growth rate + 3.6 ($r = 0.994$). Because of the small changes in breakdown rate in the marginally malnourished rats no regression analysis was made.

Table 3. Growth of muscle protein and composition throughout rehabilitation of severely malnourished rats (experiment II)

Measurements were made on Wistar rats as described for experiment I except that only the gastrocnemius muscle was examined. The fractional growth rates for the severely malnourished and the two control groups were approximate, since they were calculated from the rate of change of body weight over the period immediately before death. It was only possible to calculate the S.D. of the growth rates for the 3- and 8-day-re-feeding groups.

	Wt. (g)	RNA DNA	Protein DNA	Muscle protein (mg)	Fractional growth rate (%/day)
Protein-free diet for 30 days	56 ± 4	0.67 ± 0.11	206 ± 22	51.3 ± 4.0	-3.0
+1 day on high-protein diet	64 ± 4	0.80 ± 0.11	207 ± 17	52.3 ± 4.3	0
+3 days on high-protein diet	78 ± 6	1.48 ± 0.15	236 ± 25	59.5 ± 3.8	5.4 ± 0.8
+8 days on high-protein diet	97 ± 10	2.72 ± 0.10	267 ± 0.10	73.8 ± 6.1	6.6 ± 1.0
+14 days on high-protein diet	128 ± 11	2.67 ± 0.11	338 ± 18	115.0 ± 21.9	6.0
Weight control	110 ± 6	2.28 ± 0.12	222 ± 5	81.2 ± 3.9	5.0
Age control	205 ± 10	1.96 ± 0.26	407 ± 31	206 ± 23.5	0.7

Table 4. Fractional rate of protein synthesis (F.S.R.) and the activity of muscle RNA throughout rehabilitation of severely malnourished rats (experiment II)

Measurements were made as described in Table 2 except that the gastrocnemius muscle alone was analysed. Each group included six rats and the results are shown as means $\pm$ 1 S.D.

	F.S.R. (%/day)	Activity of RNA (g of protein/day per g of RNA)
Protein-free diet for 30 days	2.7 $\pm$ 0.9	8.0 $\pm$ 2.6
+1 day on high-protein diet	5.9 $\pm$ 0.1	15.3 $\pm$ 2.9
+3 days on high-protein diet	10.4 $\pm$ 2.0	16.6 $\pm$ 3.0
+8 days on high-protein diet	19.8 $\pm$ 2.8	19.2 $\pm$ 3.2
+14 days on high- protein diet	17.1 $\pm$ 1.7	20.3 $\pm$ 1.7
Weight control	13.8 $\pm$ 2.6	13.8 $\pm$ 3.2
Age control	7.8 $\pm$ 1.2	15.4 $\pm$ 2.5

A second consequence of hypertrophic growth is that because of protein turnover there will be a theoretical upper limit to cell size. Thus a fixed number of ribosomes will have to replace proteins degraded in an expanding protein pool and so growth will stop when the two processes are balanced. The largest possible cell would contain optimally active ribosomes and proteins with minimum rates of breakdown (in these rats about 4%/day). In practice the fall with age in the activity of the RNA means that the ultimate cell size will be smaller than would otherwise obtain. According to these arguments, then, the smaller cell size in the malnourished rats at 330 days was an inevitable result of the lower activity of the RNA (compared with the controls) since neither the breakdown rate nor the RNA/DNA ratio were significantly different from those in the well-fed rats. These arguments of course only relate to the control of hypertrophic growth. A failure of hyperplasia was an equally important component of the growth failure in the malnourished rats. However, a discussion of this is outside the scope of this paper (see Cheek *et al.*, 1971).

An elevated rate of protein breakdown also appeared to limit muscle growth during nutritional rehabilitation. In this case, because the RNA/DNA ratio was only 0.67 and the activity of the RNA was decreased, the potential at the beginning of the rehabilitation for an increase in synthesis was considerable. Consequently during the first 8 days the synthesis rate increased eightfold. Nevertheless, the breakdown rate rose to the extent that growth was limited to 6–7% per day.

To summarize, we suggest that the growth of muscle protein is limited in different ways in different situa-

Fig. 4. Growth and protein turnover rates and RNA/protein ratios in skeletal muscle of severely malnourished rats during a 2-week period of rehabilitation on a good diet

The rats are described in Table 2. Results from the control rats are indicated by the shaded columns; 1 S.D. is shown in those cases where they could be calculated.

tions. (1) On a good diet the maximum growth rate is limited by the accompanying high breakdown rate. The decline in growth rate with age results from a decline in synthesis rate, and this occurs because of a fall in activity and concentration of RNA; the latter fall being consequent on muscle cell hypertrophy. (2) On a marginally poor diet protein synthesis is limiting because of decreased ribosomal activity. To some extent maintenance of the breakdown rate at a low value can compensate for these limitations on synthesis, but this compensation is not complete. (3) In the absence of dietary protein, synthesis is limited because of decreased ribosome concentration and activity, as well as the failure of the amino acid supply.

Protein synthesis in muscle

The extent to which the rate of muscle protein synthesis can vary, as demonstrated by the results presented here, is quite remarkable, and probably unique to muscle. Although these results in no way indicate the mechanism of the changes in synthesis

rates, one general point can be made. This relates to the question of ribosome concentration as a limiting factor in the rate of protein synthesis. Apart from very acute changes in synthesis rates, alterations in ribosome concentration are quantitatively the most important component of changes in synthesis rates (Millward *et al.*, 1973). If ribosomes became rate-limiting, this would be reflected by a maximum value for the activity of RNA (synthesis rate/unit of RNA). We have never observed activities higher than the highest values reported here in muscle or in any other tissue (Millward *et al.*, 1975*b*). It is possible therefore that in the weanling well-fed rat and during rehabilitation, ribosomes were rate-limiting and the activity at that time was near maximum.

Protein breakdown in muscle

The increased breakdown rates during rapid muscle growth have been reported in other studies from our laboratory. Thus the transient rapid hypertrophy of the hemi-diaphragm which follows unilateral phrenectomy is also accompanied by increased protein breakdown (Turner & Garlick, 1974). However, other workers have reported different results. Young *et al.* (1971) reported that during rehabilitation of malnourished rats muscle protein breakdown was decreased to zero. Goldberg (1969*a*) reported that work-induced hypertrophy of the plantaris muscle resulted from a combination of increased synthesis and decreased breakdown. It is important therefore to examine possible reasons for these discrepant results.

We have calculated the breakdown rate as the difference between growth and synthesis rate. The validity of the assumptions on which the constant-infusion method is based are discussed in detail elsewhere (Aub & Waterlow, 1970; Waterlow & Stephen, 1968; Garlick *et al.*, 1974). The important point is that the method gives a direct measure of the rate of peptide-bond synthesis and although the synthesis rate is expressed as a fractional rate, no assumptions are made about the kinetics of either synthesis or breakdown. In contrast, the experiments of Young *et al.* (1971) and Goldberg (1969*a,b*), involved measurements of the decay rate of labelled proteins. Although reutilization of labelled amino acids was reported to be a minimum in each case, the calculations do rely on the assumption that muscle protein breakdown is random. We suggest that for the myofibrillar proteins, comprising two-thirds of muscle protein, this assumption may not be correct.

Myofibrillar turnover is in no way understood. Some years ago Dreyfus *et al.* (1960) reported that myosin was not degraded randomly but rather had a lifetime of 30 days. These results are often said to be erroneous because of the problem of reutilization of the labelled glycine used, and because of the apparent

abolition of lifetime kinetics when a high-protein diet was fed. The investigations of Morkin of myosin turnover in heart and skeletal muscle (Kimata & Morkin, 1971; Morkin *et al.*, 1972) do not indicate lifetime kinetics, but these experiments are still complicated by the problem of reutilization of labelled amino acids. In fact, no unambiguous study of myofibrillar protein turnover has been reported to our knowledge. However, indirect evidence suggests that myofibrillar protein turnover might be non-random.

Morkin has reported experiments which suggest that both in the diaphragm (Morkin, 1970) and heart (Morkin, 1974) myofibrils are renewed by the addition of newly synthesized myofilaments to the periphery of the myofibril. Further, Rabinowitz and co-workers (Martin *et al.*, 1974) have suggested that their data on the incorporation and decay of labelled leucine in cardiac myosin together with other data on the presence of highly labelled preferentially extractable myosin in muscle (Etlinger *et al.*, 1974; Etlinger, 1973) suggest that newly synthesized myosin may exist as a separate pool from the bulk of the myofibril. If newly synthesized myofilaments are added to the periphery of the myofibril then turnover cannot be completely random.

The implication of non-random myofibrillar turnover is that the loss of labelled amino acids after a pulse dose may vary according to the subsequent treatment. For example, during starvation, when the myofibrillar diameter falls (Goldspink, 1965) peripheral highly labelled filaments would be degraded with an acceleration of loss of radioactivity. In a previous study (Millward, 1970*b*) we observed that between 3 and 7 days after labelling in starved rats there was an accelerated loss of radioactivity from muscle myofibrillar proteins. This would indicate degradation of a highly labelled protein pool.

The retention of radioactivity during rapid growth (Goldberg 1969*a*; Young *et al.*, 1971) when breakdown is increased, according to our results, is more difficult to reconcile. One possibility is that the discrepancy may result from the way in which myofibrils proliferate. According to Goldspink (1970, 1972) during muscle growth large-diameter myofibrils split longitudinally to form daughter myofibrils. If the splitting is not completely conservative so that myofibrillar portions or individual myofilaments become unattached during the split then these may be degraded. Thus rapid growth and the consequent increased myofibrillar splitting would result in increased protein turnover. Further, the peripheral myofilaments, highly labelled after a radioactive pulse, may not be included in this increased turnover. Thus the loss of radioactivity would underestimate the breakdown rate. Of course, this is only one of several possibilities, but it seems to us that the myofibrillar matrix that extends the complete length of the muscle fibre, with force transmission absolutely

dependent on its integrity, is highly unlikely to have the same turnover characteristics as individual enzyme proteins in, for example, liver cytoplasm.

These results in no way prejudice the case for muscle protein as a mobile protein reserve for gluconeogenesis as demonstrated, for example, by Cahill (1970). Muscle protein does by some unknown mechanism turn over continuously, and because of the extreme sensitivity of muscle synthesis to dietary and hormonal changes, which may be unique to this tissue, amino acids can be liberated at all times by a decrease in the synthesis rate.

Finally, these results suggest a possible explanation for the very large discrepancy between the energy cost of protein deposition during growth, determined experimentally (Kielenowski, 1972) and the cost calculated from the requirements of 4 mol of ATP and of GTP/mol of peptide bond. If muscle growth *per se* increased protein turnover, then the energy cost of the deposition of protein must include a component to account for this increased turnover.

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