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The Relative Importance of Muscle Protein Synthesis and Breakdown in the Regulation of Muscle Mass

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The effects of growth-suppressing and muscle-wasting treatments on muscle protein turnover and amino acid concentrations were determined *in vivo*. All treatments depressed protein synthesis and some treatments depressed protein breakdown. Only prolonged starvation increased protein breakdown. Muscle protein mass is regulated primarily through alterations in protein synthesis in all except emergency conditions. The increased concentrations of the branched-chain amino acids indicate that they are unlikely to be involved in this regulation.

Losses of skeletal-muscle protein are an important component of whole-body nitrogen losses in such conditions as starvation (Cahill, 1970) and post-operative trauma (Cuthbertson *et al.*, 1972). Although it has been shown that such catabolism may result from a suppression of protein synthesis (O'Keefe *et al.*, 1974) rather than the more traditional mechanism of increased protein breakdown, few measurements of muscle protein turnover *in vivo* in such conditions have been reported. We have investigated therefore the changes in muscle protein synthesis and breakdown in rats receiving a range of inadequate diets and in diabetic, hypophysectomized and glucocorticoid-treated rats. All these conditions stop growth and some induce rapid loss of muscle protein. We have measured rates of protein synthesis and breakdown *in vivo*, estimated ribosomal activity *in vivo*, and measured concentrations of methionine and the branched-chain amino acids, since the latter amino acids have been cited as controlling factors in muscle protein turnover (Fulks *et al.*, 1975; Buse & Reid, 1975).

Materials and Methods

All measurements were made on male Wistar albino rats (weighing 80–150g, unless otherwise stated). All rats were fed on a stock cubed diet, except for those starved or those fed on the protein-free diet (Millward *et al.*, 1974). The hypophysectomized rats (Anglia Laboratory Animals, Alconbury, Huntingdon, U.K.) were fed *ad lib.* but their food intake was measured. A group of unoperated controls were fed on a similar amount of the stock diet, and since this was less than a normal intake these rats were designated the low-energy group. Diabetes was induced by the injection of 100mg of streptozotocin/kg body wt. 6 days before measurements were made, and monitored by measurements of blood glucose and insulin concentrations. Rats treated with glucocorticoids were given daily intraperitoneal injections of 5mg of triamcinolone acetonide (E. R. Squibb and Sons, Speke, Liverpool, U.K.)/kg body wt.

The rate of protein synthesis was measured *in vivo* by the constant-intravenous-infusion method (Waterlow & Stephen, 1968; Garlick *et al.*, 1974) as described previously (Millward *et al.*, 1975). Measurements were made on the combined quadriceps and gastrocnemius muscles from one hind-limb, and these two muscles from the other limb were analysed for free amino acids, RNA and non-collagen protein as previously described (Millward *et al.*, 1974). Groups of rats in addition to those infused were also killed throughout the various treatments to determine the rate of change of total muscle non-collagen protein at the time of infusion. The breakdown rate was then determined by subtracting the fractional rate of net change from the fractional synthesis rate (Millward *et al.*, 1975).

Results and Discussion

Overall effects of the treatments

All of the treatments described in Table 1 resulted in a decrease in the rate of protein synthesis, but there were variable changes in the rate of protein breakdown. However, the results fall naturally into three groups, and have been arranged as such in Table 1. In the first group (1 day protein-free diet, 10 days low-energy intake and 2 days glucocorticoids) the treatments abolished growth by suppressing protein synthesis. No measurable net

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Table 1. *Fractional rates of protein synthesis and of protein breakdown and the activity of muscle RNA from well-fed and treated rats*

Measurements were made on the combined gastrocnemius and quadriceps muscles (four to eight rats). Rates of protein synthesis were measured by the constant intravenous infusion of [^{14}C]tyrosine. The activity of RNA was determined by dividing the protein fractional synthesis rate by the RNA/protein ratio. The breakdown rate was determined as the difference between the synthesis rate and the rate of net change. Values are means±S.D.

Treatment	Protein fractional synthesis rate (%/day)	(% of control)	Activity of RNA (g of protein/day per g of RNA)	Protein fractional breakdown rate (%/day)	(% of control)
(1) Control	18.7 (2.0)		15.1 (2.3)	11.0	
Protein-free diet (1 day)	9.8 (1.1)	52	9.3 (0.8)	9.8	89
Control	12.9 (1.7)		17.7 (3.2)	8.7	
Low energy intake (10 days)	7.1 (0.9)	55	7.4 (1.3)	7.1	82
Control	12.9 (1.6)		9.2 (1.4)	8.7	
Glucocorticoids (2 days)	10.8 (1.7)	84	8.4 (1.0)	11.0	126
(2) Control	12.9 (1.6)		9.2 (1.4)	8.7	
Diabetes	4.8 (0.8)	37	6.0 (1.0)	4.8	55
Control	12.9 (1.7)		17.7 (3.2)	8.7	
Hypophysectomy	5.8 (1.3)	45	6.8 (1.5)	5.8	67
Control	18.7 (2.0)		15.1 (2.3)	11.0	
Protein-free diet (9 days)	5.1 (1.5)	27	10.3 (1.6)	5.1	46
Protein-free diet (30 days)	2.7 (0.9)	14	8.0 (2.6)	5.7	52
Control	16.5 (1.7)		18.2 (3.7)	8.9	
Starved (2 days)	6.9 (0.9)	42	8.1 (0.8)	6.9	77
(3) Control (100g)	16.5 (1.7)		18.2 (3.7)	8.9	
Starved (4 days)	4.7 (0.5)	28	8.1 (0.8)	18.7	210
Control (400g)	4.5 (0.6)		11.5 (1.1)	3.7	
Starved (4 days)	2.6 (0.3)	57	9.0 (0.8)	6.4	172
All controls (range of means)			9.2–18.2		
All treatments (range of means)			6.0–10.3		

catabolism was observed in any case, and the rate of protein breakdown was only marginally altered. Although the glucocorticoid-treated rats are also included in this group, the mechanism of growth suppression was less obvious, however. This was because there were small changes in rates of protein synthesis and breakdown.

The characteristic feature of the second group of treatments was a more extensive suppression of protein synthesis and breakdown. In most cases no protein was lost from the muscles, but in the rats fed on the protein-free diet for 30 days net catabolism was observed at the rate of 3%/day. This loss resulted from a greater fall in the synthesis rate than in the breakdown rate.

It might be thought surprising that none of the treatments included in this group induced rapid loss of muscle protein. However, the hind-limb muscles may be protected against undue net catabolism because of stimulated locomotor activity in malnourished animals compared with other skeletal muscles with a lower degree of functional utilization (Wechsler, 1966).

The third group includes both young (100g) and adult (400g) rats that were starved for 4 days. The young rats were rapidly losing protein at the rate of 14%/day, and this was achieved by a marked depression of synthesis coupled to an increase in the breakdown rate. In the adult rats the response was qualitatively the same, but the absolute magnitude of the rates of synthesis, breakdown and net change were less in each case.

Protein synthesis

These results illustrate the sensitivity and the range of variability of protein synthesis in muscle (Garlick *et al.*, 1975). This variability results from changes in both RNA content and activity (Millward *et al.*, 1973). Since most muscle RNA is ribosomal, measurement of RNA activity indicates ribosomal activity or translation rates (Millward *et al.*, 1973). Table 1 shows the activity of RNA in the various groups. It is apparent that there were considerable variations in RNA activity between the control groups. Although there were no other obvious differences between control groups, we consider that these variations are real and indicate the normal range of activity of the protein-synthetic apparatus in muscle. The highest activity (18.2g of protein/day per g of RNA) is similar to the value observed in rapidly growing rats (Millward *et al.*, 1975).

Table 2. *Concentrations of methionine and the branched-chain amino acids in muscle*

Measurements were made on pooled supernatants from the combined gastrocnemius and quadriceps muscles (from four to eight rats) homogenized in 5% (w/v) sulphosalicylic acid.

	Concentration of amino acids					
	(μmol/g)	(% of control)				
	Controls	Diabetic	Hypophysectomy	Low energy intake (10 days)	Starvation (2 days)	Glucocorticoids (2 days)
Methionine	0.055	222	133	200	380	200
Leucine	0.133	408	165	256	420	150
Isoleucine	0.090	399	122	211	480	147
Valine	0.176	435	114	193	150	143

All of the treatments significantly decreased the RNA activity compared with that of the appropriate control group. However, Table 1 shows that there was some degree of overlap between the complete range of treated and control animals. Thus the rats fed on the protein-free diet for up to 9 days maintained ribosomal activities at the lower end of the normal range. The lowest activities were observed in the rats fed on smaller amounts of food (low energy), the diabetic rats and the hypophysectomized rats. It appears, then, that in skeletal muscle there is an approximate threefold range in ribosomal activity ranging from 6 in diabetic rats to 18 (g of protein/day per g of RNA) in rapidly growing rats. Presumably the upper limit indicates maximal rates of initiation and elongation. The translation rate can be estimated from this value if it is assumed that ribosomal RNA accounts for 80% of total tissue RNA and that 80% of ribosomes are translating (as in the liver; Scornik, 1974). Thus, assuming mol.wts. of amino acids and ribosomal RNA to be 110 and 2.45×10^6 respectively, an activity of 18.2g of protein/day per g of RNA is equal to a translation rate of 7.3 amino acids/s per ribosome, similar to the rate observed in liver (Scornik, 1974). Although this value is obviously approximate, it is liable to be, if anything, an underestimate, since ribosomal RNA is unlikely to be much more than 80% of total tissue RNA.

These studies *in vivo* do not, however, indicate the mechanism of the changes in muscle protein synthesis. That insulin and growth hormone are both controlling hormones is indicated by the fact that activities are lowest in diabetic and hypophysectomized rats. The role of glucocorticoids in muscle protein metabolism is difficult to evaluate in these studies, since the administration of glucocorticoids to well-fed rats induces severe hyperinsulinaemia (Millward *et al.*, 1976). This means that there is an unusual situation *in vivo* in which potentially catabolic and anabolic hormones are simultaneously present.

High concentrations of the branched-chain amino acids in general (Fulks *et al.*, 1975) and leucine in particular (Buse & Reid, 1975) have been reported to stimulate muscle protein synthesis and inhibit breakdown. A regulatory role for these amino acids in muscle is attractive, because the tissue is an important site of their oxidation (Johnson *et al.*, 1961; Manchester, 1965).

However, the way in which the concentrations of these amino acids in skeletal muscle change in various conditions does not support such a regulatory role. Thus during starvation the concentration of the branched-chain amino acids and methionine increases in skeletal muscle *in vivo* (Millward *et al.*, 1974), whereas in the perfused hemicorpus preparation the insulin-stimulated increase in protein synthesis is accompanied by falls in the concentrations of all these amino acids (Jefferson *et al.*, 1974). The results in Table 2 show that, in all the treatments in which amino acid concentrations were measured, increases in branched-chain amino acid and methionine concentrations were observed. Particularly dramatic increases were apparent in the diabetic rats and starved rats. We have previously argued that such changes would be predicted because the relative abundance of these particular amino acids in the free pool is much less than their relative abundance in protein (Millward *et al.*, 1973). Thus their concentration will fall as they become rate-limiting when synthesis is stimulated, and rise during net catabolism. The fact that marked loss of muscle protein was not observed in any of the treatments listed in Table 2 indicates the sensitivity of the changes in the concentrations of these amino acids as an indicator of the net status of muscle protein turnover.

Protein breakdown

Although there were marked decreases in the synthesis rates, rapid losses of protein did not always

occur because decreases also occurred in the breakdown rate. Even when protein was lost at the rate of 3%/day in the rats fed on the protein-free diet for 30 days, the breakdown rate was less than in the control group. Similar results are indicated in man during starvation (Young *et al.*, 1973), and in rats fed on a protein-deficient diet for 14 days (Haverberg *et al.*, 1975), when 3-methylhistidine excretion falls, indicating diminished muscle protein breakdown.

Thus the increases in the breakdown rate in muscle after 4 days' starvation could be seen as an emergency response. Bird (1975) has reported that secondary lysosome formation and the initiation of autophagy in rat muscle can be observed during the fifth and sixth days of starvation.

It is not immediately apparent why the breakdown rate should fall with the synthesis rate, although there will be an obvious saving of energy as a result of the diminished turnover. Protein breakdown rates in rat muscle are highest during rapid growth, and fall as the growth rate falls (Millward *et al.*, 1975). If increased protein breakdown is a necessary accompaniment to growth, any interruption of growth might be expected to decrease the rate of protein breakdown. Certainly, as shown by the present results in 400g adult rats, in which muscle growth has virtually stopped, turnover is slow.

The decreased rates of protein breakdown in the diabetic rats, 2-day-starved rats and protein-deficient rats might be thought surprising in view of the evidence that muscles incubated *in vitro* without additions of insulin or substrates exhibit increased rates of protein breakdown (Fulks *et al.*, 1975). *In vivo*, however, as we show here, concentrations of rate-limiting amino acids are increased, and this may protect against increased lysosomal activity, as is observed in liver (Mortimore & Neely, 1975). Also, in the diabetic rats the plasma insulin concentration was not insignificant (8 μunits/ml).

We can conclude, then, that, in skeletal muscle, regulation of protein content is achieved through alterations of the synthesis rates as the primary response, by mechanisms that are unlikely to involve the branched-chain amino acids. Increases in the breakdown rate during net catabolism appear to occur only in response to exceptional situations.

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