The Effects of Food Deprivation on Protein Turnover and Nucleic Acid Concentrations of Active and Immobilized Extensor Digitorum Longus Muscles of the Rat

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Deprivation of food caused significant changes in the weight, protein content, protein turnover and RNA concentrations of the extensor digitorum longus muscle. Simultaneous immobilization to render the muscle inactive did not make the tissue any more susceptible to the effects of starvation. In contrast, immobilization in a stretched state resulted in less muscle wasting after deprivation of food.

Starvation or prolonged feeding on a low-protein diet is known to cause an appreciable loss of the body's weight and protein. Individual tissues, however, show a disproportionate response to malnutrition, with skeletal muscle losing weight and protein to a very marked degree (Waterlow & Stephen, 1966). This is not surprising since skeletal muscle is the largest single tissue of the body and its protein represents a large potential supply of amino acids for use as substrate for gluconeogenesis. However, not all muscles undergo the same degree of atrophy after altering the animal's state of nutrition (Dickerson & McCance, 1960. Generally, phasic muscles are more affected than tonic muscles.

The present investigation was undertaken to examine how starvation affects protein metabolism of the extensor digitorum longus muscle. Secondly, protein turnover (measured *in vitro*) was compared in active and immobilized extensor digitorum longus muscles to determine if contractile activity is a factor influencing the tissue's responsiveness to starvation.

Experimental

All experiments involved the use of male Charles River rats (initially 50–55 g body wt.). The foot of one hind limb was immobilized in one of two positions (Goldspink, 1977a). When the ankle was restrained in a dorsiflexed position the extensor digitorum longus muscle was maintained in a fully shortened state, but when immobilized in extension the extensor digitorum longus muscle was held in a lengthened position. Control animals were returned to their cages and fed *ad libitum*. Starved animals, receiving only water, were individually housed in cages with a mesh floor. Then 46 h later all animals were killed by cervical dislocation. The appropriate internal-control and immobilized extensor digitorum longus muscles were rapidly dissected out and

average rates of protein synthesis and protein breakdown were measured in these isolated muscles by the methods of Fulks *et al.* (1975). These methods involve the amino acid tyrosine, since it is neither synthesized nor degraded by skeletal muscle. That is, protein synthesis was measured as the incorporation of tyrosine into muscle proteins after a 3 h incubation in 3 ml of oxygenated (O₂/CO₂, 19:1) Krebs–Ringer bicarbonate (Deluca & Cohen, 1964), containing 10 mM-glucose, 5 times the normal concentration of all plasma amino acids except tyrosine, 0.05 μ Ci of L-[U-¹⁴C]tyrosine (sp. radioactivity 483 mCi/mmol; The Radiochemical Centre, Amersham, Bucks., U.K.) and L-tyrosine hydrochloride (0.1 mM). The [¹⁴C]-tyrosine measured in protein was divided by the specific radioactivity of the intracellular tyrosine pool, to give the number of moles of tyrosine incorporated into muscle proteins (Fulks *et al.*, 1975). Protein degradation was determined independently of synthesis by measuring the release of tyrosine (Waalkes & Udenfriend, 1957) into intracellular amino acid pools and the surrounding medium after a 3 h incubation. Cycloheximide (0.5 mM) was added to this medium to block protein synthesis, thus preventing any reutilization of the released tyrosine.

Results and Discussion

The extensor digitorum longus muscle of one limb was immobilized in either a shortened (Table 1), or a lengthened (Table 2), position immediately before 46 h of continued normal feeding or food deprivation. The effects of food deprivation were then studied on the immobilized muscles and their internal controls from the contralateral limb. The use of such internal controls has previously been validated for these conditions of immobilization (Goldspink, 1978).

The immobilization of the extensor digitorum longus muscles (in a shortened position) in fed rats produced significant decreases in the rates of protein synthesis and increases in the rates of protein breakdown (Table 1). These changes, however, were probably not sufficiently rapid in onset (Goldspink, 1977a) to cause statistically significant decreases in muscle weight and protein content (Table 1). Simultaneous food deprivation induced even greater changes in the rates of protein synthesis and degradation within the immobilized muscle (Table 1). However, very similar changes also occurred within the control extensor digitorum longus muscles after this period of starvation. The animals lost $27 \pm 0.8\%$ ($P < 0.001$) of their body weight, and the internal control muscles were smaller (30% , $P < 0.001$) and contained less protein (9% , $P < 0.005$) after food deprivation. These changes in the internal control extensor digitorum longus muscle after deprivation of food correlated with highly significant (i.e. $P < 0.005$) decreases in the rates of protein synthesis (62 or 73% per g of muscle or per whole muscle respectively) and increases in the rates of protein catabolism (101 or 40% per g of muscle or per whole muscle respectively). Hence the effects of this form of immobilization in the fed and starved states were very similar (i.e. internal comparisons;

Table 1), suggesting that both active and inactive muscles were equally susceptible to the catabolic effects of starvation.

These induced changes in protein synthesis and protein degradation, as measured by these techniques *in vitro*, probably provide a good, if indirect, indication of the events taking place in the intact animal (Goldspink, 1978). These findings are further supported by studies *in vivo* that report similar decreases in the protein synthetic rates of muscle after starvation (Millward, 1970; Garlick *et al.*, 1973; Millward *et al.*, 1973) or feeding animals on a low-protein diet (Waterlow & Stephen, 1967, 1968; Young *et al.*, 1971). The decreases in protein synthesis per unit RNA after immobilization in this shortened position, or after deprivation of food (Table 1), probably indicate decreased efficiencies of muscle ribosomes in the translation process (Millward *et al.*, 1973). These results are supported by Young & Alexis (1968), who found that after feeding rats on a low-protein diet muscle ribosomes possessed a smaller proportion of polyribosomes and were less active in incorporating amino acids into protein. Much less attention has been directed at determining the effects of food deprivation on the protein-degradative rates of muscle. In agreement with this study (Table 1), a few workers (Millward, 1970; Young *et al.*, 1971;

Table 1. Effects of 46h of starvation on the wet weight, protein content, protein turnover and nucleic acid concentrations of internal control and immobilized (unstretched) extensor digitorum longus muscles

The extensor digitorum longus muscle of one limb was immobilized in a fully shortened position (Goldspink, 1977a). Animals were subsequently fed normally or deprived of all food for 46h. Rates of protein synthesis and breakdown were measured in the isolated immobilized or internal control tissues by the method of Fulks *et al.* (1975). The extraction and assay procedures for RNA or DNA (Goldberg & Goldspink, 1975) and protein (Lowry *et al.*, 1951) are described elsewhere. Each value is the mean $\pm$ S.E.M. of measurements made on at least six immobilized or control muscles. The Student's *t* test was used for all statistical analyses: * $P < 0.01$; ** $P < 0.025$.

	Fed rats			Starved rats		
	Control muscle	Immobilized muscle	Percentage change	Control muscle	Immobilized muscle	Percentage change
Wet wt. (mg)	22 ± 0.9	20 ± 0.2	-10	16 ± 0.5	15 ± 0.6	-2
Protein (mg)	4.7 ± 0.1	4.3 ± 0.1	-9**	3.3 ± 0.1	3.2 ± 0.05	-6
Protein synthesis						
nmol of tyrosine incorporated/muscle	11 ± 0.1	8.7 ± 0.3	-28*	3.1 ± 0.2	2.4 ± 0.2	-21*
nmol of tyrosine incorporated/g of muscle	521 ± 7	434 ± 17	-17*	198 ± 10	158 ± 10	-20*
nmol of tyrosine incorporated/ μ g of RNA phosphorus	2.6 ± 0.06	2.2 ± 0.08	-15*	1.1 ± 0.06	0.8 ± 0.06	-24*
Protein breakdown						
nmol of tyrosine released/muscle	9.2 ± 0.2	14 ± 0.8	+51*	13 ± 0.5	17 ± 0.6	+32*
nmol of tyrosine released/g of muscle	416 ± 22	656 ± 52	+58*	836 ± 32	1095 ± 32	+31*
RNA phosphorus						
μ g/muscle	4.6 ± 0.1	4.0 ± 0.02	-12	3.1 ± 0.1	3.0 ± 0.1	-4
μ g/g of muscle	208 ± 5	201 ± 1	-3	198 ± 7	192 ± 5	-3
DNA phosphorus						
μ g/muscle	2.9 ± 0.1	3.0 ± 0.1	+4	2.8 ± 0.1	2.8 ± 0.1	+1
μ g/g of muscle	131 ± 4	149 ± 4	+14*	178 ± 4	179 ± 5	+1
RNA/DNA ratio	1.6 ± 0.04	1.4 ± 0.04	-14*	1.1 ± 0.04	1.1 ± 0.04	-3

Fulks *et al.*, 1975) have reported increased rates of protein catabolism after starvation or protein malnutrition. These increased rates of protein degradation are in keeping with measured increases in the autolytic activity of muscles from starved animals (Pennington, 1977).

Changes in total RNA were only slight after immobilization, but were appreciable (33%; $P < 0.001$) after starvation (Table 1; Young *et al.*, 1971; Goldberg & Goldspink, 1975). Although muscle DNA synthesis is suppressed by immobilization (Goldspink, 1977a) or starvation (Goldberg & Goldspink, 1975), no change in DNA content/muscle was found over this short period of time. Since DNA content/g of muscle increased in both experimental situations (Table 1, and 36% after starvation; $P < 0.001$), the degree of inhibition of DNA synthesis must be less than the rate of muscle wasting.

In contrast to the above, immobilization in a lengthened position led to a greater growth of the extensor digitorum longus in fed rats (Table 2 and Goldspink, 1977a). This stretch-induced growth correlates with similar size increases in both protein synthesis and breakdown (Table 1). However, since the basal rates are such that synthesis exceeds breakdown (Table 1), and the changes in breakdown are slower in onset than those of synthesis (Goldspink, 1977a), the net effect is anabolic. The effects of starvation on the internal control muscles were again similar to those discussed above (Table 1).

However, the extensor digitorum longus muscle immobilized in this lengthened state lost significantly less weight, protein, RNA and DNA than did the internal control muscle after starvation (Table 2). Hence the growth-promoting influences associated with immobilization in this position were still effective despite the surrounding catabolic environment. The slightly greater growth of the immobilized extensor digitorum longus muscle in starved animals, compared with fed ones, may be due to the absence of the induced increase in protein breakdown, which occurred in the fed state (Table 2).

Starvation for 46 h was found to induce a significantly greater atrophy of the extensor digitorum longus muscle (30%) than the soleus (12%) muscle. This is consistent with the idea that the greater activity found in the tonic soleus muscle may protect this tissue to a greater extent from various catabolic influences, including starvation. This hypothesis concerning muscular activity was not supported by the first part of this study, because both inactive and active extensor digitorum longus muscles were found to be equally susceptible to the effects of starvation (Table 1). It is, however, possible that the starved rats were generally less mobile than the fed ones. If this is so, the activity in the internal control muscles may not be strictly comparable in these two nutritional states. Hence, less activity would in fact be removed by immobilization in the starved state, and the internal comparisons would tend to under-

Table 2. Effects of 46 h of starvation on the wet weight, protein content, protein turnover and nucleic acid concentrations of internal-control and immobilized (stretched) extensor digitorum longus muscles

In these experiments the extensor digitorum longus muscle of one limb was immobilized in a lengthened and stretched state (Goldspink, 1977a). In all other aspects identical procedures were used to those described in Table 1. * $P < 0.01$; ** $P < 0.025$.

	Fed rats			Starved rats		
	Control muscle	Immobilized stretched muscle	Percentage change	Control muscle	Immobilized stretched muscle	Percentage change
Wet wt. (mg)	23 ± 0.6	25 ± 0.7	+9*	16 ± 0.5	19 ± 0.7	+16*
Protein (mg)	4.9 ± 0.1	5.1 ± 0.2	+5	3.2 ± 0.1	3.5 ± 0.1	+12**
Protein synthesis						
nmol of tyrosine incorporated/muscle	10 ± 0.6	16 ± 1.1	+57*	2.4 ± 0.2	4.7 ± 0.2	+92*
nmol of tyrosine incorporated/g of muscle	422 ± 23	624 ± 44	+48*	148 ± 11	243 ± 9	+64*
nmol of tyrosine incorporated/μg of RNA phosphorus	2.2 ± 0.1	2.8 ± 0.2	+27*	0.8 ± 0.08	1.3 ± 0.04	+60*
Protein breakdown						
nmol of tyrosine released/muscle	8.4 ± 0.5	13 ± 1.2	+58*	13 ± 0.7	13 ± 0.6	-3
nmol of tyrosine released/g of muscle	354 ± 23	529 ± 49	+49	786 ± 44	694 ± 32	-12
RNA phosphorus						
μg/muscle	4.5 ± 0.05	5.5 ± 0.1	+23*	2.6 ± 0.2	3.6 ± 0.1	+39*
μg/g of muscle	188 ± 2	219 ± 5	+17*	159 ± 10	189 ± 5	+19**
DNA phosphorus						
μg/muscle	3.4 ± 0.1	3.3 ± 0.05	-10	2.8 ± 0.03	2.8 ± 0.04	+1
μg/g of muscle	144 ± 4	131 ± 2	-9*	170 ± 2	147 ± 2	-14*
RNA/DNA ratio	1.3 ± 0.05	1.7 ± 0.04	+32*	1.0 ± 0.03	1.3 ± 0.03	+31*

estimate the real effects. Such lowering of the activity in the control tissues would also have the added effect (Goldspink, 1977b) of exaggerating the influence of food deprivation on protein turnover. Supporting the hypothesis, however, was the finding that the second immobilization procedure, which increases the growth-promoting influences in the extensor digitorum longus muscle, appeared to make this tissue more resistant to the effects of starvation (Table 2).

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