PROTEIN SYNTHESIS DURING WORK-INDUCED GROWTH OF SKELETAL MUSCLE

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The capacity of skeletal and cardiac muscle to increase in size in response to increased physiological demand is well known. However, little is known of either the biochemical mechanisms involved in this compensatory hypertrophy or the cellular and physiological changes resulting from this growth process. Studies of protein synthesis during work-induced hypertrophy are relevant to such questions, since over 80% of the dry weight of muscles is protein (1). The present in vivo experiments compare the incorporation of amino acids into proteins by hypertrophying and control muscles, and they attempt to define the types of proteins made during compensatory growth.

In order to study work-induced hypertrophy as an isolated process, we carried out these experiments with hypophysectomized animals. Although removal of the pituitary leads to cessation of normal developmental growth, compensatory hypertrophy of muscle occurs normally in such animals (2). Rapid compensatory growth of the soleus and plantaris muscles of one hind limb of the rat can be induced by severing the connections of the gastrocnemius to the Achilles tendon on that side (2, 3). Loss of this muscle imposes a greater work load on the remaining extensors of the ankle, and within 5 days the soleus of that limb increases in mass by 40% and the plantaris by about 20%. In the present experiments, rats received repeated injections of leucine-^{14}C after tenotomy of the gastrocnemius, and the extent of labeling of proteins in the hypertrophied and contralateral muscles was measured at the termination of the compensatory growth.

METHODS

Male rats weighing between 110 and 125 g were obtained from the Charles River Laboratories (Wilmington, Mass.) and were studied 5–7 wk after hypophysectomy. Animals that gained weight or showed testicular development during this period were discarded. Tenotomy of the gastrocnemius and contralateral sham operations were performed as described previously (2, 3). 3 μc of uniformly labeled leucine-^{14}C (1.89 mc/mg) were injected subcutaneously in 0.3 ml of physiological saline on 5 successive days. The first injection was given 12 hr after operation, and the rats were sacrificed 24 hr after the final injection, when compensatory growth had reached its maximal extent (2, 3).

The soleus and plantaris muscles were excised, weighed on a Roller Smith (Bethlehem, Pa.) torsion balance, and minced with a scissors. To insure uniform recovery of proteins from muscles of different sizes, we took samples weighing about 50 mg from each muscle. Measurements of radioactivity in counts per minute per milligram were then multiplied by the weights of the entire soleus or plantaris for total counts per muscle. In control experiments, replicate samples of the plantaris were found to be equally labeled.

The minced tissues were added to 2 ml of a cold, low ionic strength buffer (0.01 phosphate, pH 7.4) and were homogenized with a Kontes Duall tissue grinder. The homogenates were transferred to centrifuge tubes, and the grinding apparatus was rinsed with an additional milliliter of buffer. The soluble (sarcoplasmic) proteins were separated from the insoluble (myofibrillar) fraction by centrifugation at 1,000 *g* for 20 min. The proteins in the supernatant were precipitated by addition of 3 ml of cold 20% trichloroacetic acid, and the myofibrillar fraction was resuspended in 3 ml of the buffer. The tubes were centrifuged, and the supernatants were discarded. The precipitates were washed twice with cold 5% trichloroacetic acid and once with a 1:1 ethanol–ether solution. They were then dissolved in Nuclear-Chicago Solubilizer (Nuclear-Chicago Corporation, Des Plaines, Ill.) at 60° with occasional shaking. The samples were transferred quantitatively to vials for liquid scintillation counting in a Packard Tricarb Counter (Packard Instrument Co., Downers Grove, Ill.). The scintillation fluid was composed of 4 g of 2,5-bis [2-(5-tert-butylbenzoxazolyl)] thio-

phene (BBOT) and 600 ml of absolute ethanol per liter of toluene.

Incorporation of leucine-^{14}C into various sarcoplasmic fractions was determined after differential centrifugation. In each experiment the soluble proteins from three control and three hypertrophied muscles were pooled. Mitochondria and calcium-binding grana were isolated by centrifugation for 1 hr at 8,000 and 30,000 *g*, respectively (14). The supernatant was centrifuged for 2 hr at 100,000 *g* in a Model L Spinco Ultracentrifuge for sedimentation of microsomal components. All the pellets were resuspended in 3 ml of the buffer, and 5 mg of egg albumin were added to each as a carrier. 3 ml of 20% trichloroacetic acid were then added to each sample and to the supernatant. The precipitates were washed and prepared for counting as described above.

For characterization of the radioactive proteins in the insoluble fraction, myofibrillar and collagenous materials were separated according to the method of Hellander (7), and the counts in each component were assayed in the usual manner. The amount of leucine-^{14}C in actomyosin was estimated after extraction by an adaptation of the procedure of Seidel et al. (16). Pooled homogenates of three control and three hypertrophied muscles were divided into two equal portions, and each was centrifuged. The counts in the insoluble fraction in one tube were measured as already described; in the other, the precipitate was extracted for 2 hr with 12 ml of 0.6 M KCl, 0.1 M phosphate buffer (pH 7.0) with constant shaking. This suspension was then centrifuged, and the supernatant was dialyzed overnight against 2 liters of the low ionic strength buffer. The precipitates formed in the dialysis sac were transfered quantitatively to centrifuge tubes. Following centrifugation, the precipitates were washed, dissolved, and counted as above.

RESULTS

6 days after unilateral tenotomy of the gastrocnemius, the soleus of the operated limb had increased in weight by 36 ± 4% and the plantaris by 15 ± 2% (Fig. 1). In all animals studied, the growing muscles incorporated more leucine-^{14}C into protein than did the controls. No differences were found between contralateral muscles of animals which received bilateral sham operations. These results are summarized in Fig. 1, in which the relative increase in leucine incorporation by the hypertrophying muscle (expressed as per cent of its contralateral control) is plotted against the relative gain in muscle weight. Values for total incorporation are the sums of the counts measured separately in the soluble and myofibrillar fractions. Data for both the soleus and plantaris fall upon the same straight line, even though the soleus consistently grew twice as much as the plantaris, and even though the control soleus incorporated more amino acids into protein (per milligrams of tissue) than did the control plantaris (4). In both instances, the increase in muscle weight was directly proportional to the increase in amino acid incorporation and presumably protein synthesis. These

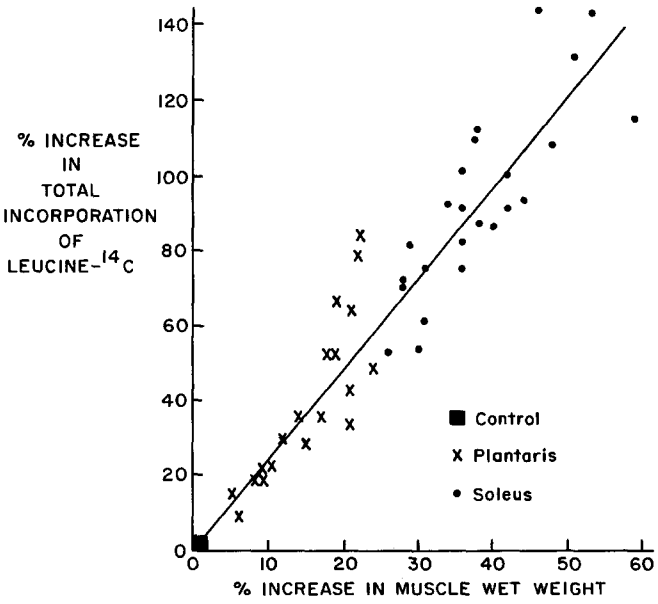

FIGURE 1 Relationship of total incorporation of leucine-^{14}C to gain in weight by hypertrophied muscle. Net growth and amino acid incorporation are both expressed as per cent increase above that found in muscles of contralateral, sham-operated limb. All measurements were made 6 days after tenotomy of the gastrocnemius in animals injected dialy with leucine-^{14}C. Control animals received no operations or received sham operations on both limbs.

results based on measurements of wet tissue weight are also valid for dry weight, since the percentage of water in the muscles does not change during hypertrophy (2).

Although greater incorporation of leucine-^{14}C occurred into both soluble and myofibrillar proteins (Table I), the ratio of myofibrillar to soluble counts did not change with hypertrophy. In other words, the relative increase in incorporation during hypertrophy was similar in the two fractions. The appearance of ^{14}C in soluble and myofibrillar proteins in the hypertrophying soleus was also measured on the first, second, and third days after tenotomy of the gastrocnemius. Again, incorporation of leucine-^{14}C increased proportionately in both fractions on each day of growth. These findings suggest that the growing muscle synthesized fibrillar and soluble proteins in approximately the same proportions as in the nongrowing muscle.

The assumption that all radioactive material insoluble at low ionic strength is in myofibrillar proteins was tested in six animals by separation of collagenous and myofibrillar proteins (7). In each case, 92% or more of the radioactivity of the insoluble fraction was extracted as myofibrillar. Furthermore, at least 71% of the labeled proteins of the insoluble fraction had solubility properties similar to those of actomyosin (Table II). Since the percentage of radioactivity extracted as actomyosin was the same in hypertrophied and control muscles, actomyosin must have been produced in increased amounts during hypertrophy.

For determination of whether any sarcoplasmic component was produced disproportionately during compensatory growth, the amount of leucine-^{14}C in various centrifugal fractions was examined (14). Table III compares the distribution of sarcoplasmic radioactivity found in mitochondria, calcium-binding grana, microsomes, and soluble proteins of hypertrophied and control muscles. The relative amounts of label in these fractions were quite uniform in the different experiments No significant differences were seen in the distributions of label in hypertrophied and control soleus muscles, even though the total incorporation in the former was much greater. Similar findings were made on the plantaris, in which the distribution of

TABLE I

Relative Incorporation of Leucine-^{14}C into Soluble and Fibrillar Proteins during Hypertrophy of Soleus

	Total soluble cpm per muscle	Total myofibrillar cpm per muscle	Average ratio of myofibrillar to soluble cpm*
Hypertrophied	1092 ± 67	1865 ± 165	1.72 ± 0.06
Control	632 ± 32	1015 ± 68	1.67 ± 0.05
Average ratio of cpm in contralateral muscles*	1.74 ± 0.08	1.82 ± 0.08	

All measurements were made 6 days after tenotomy of the gastrocnemius.
Values are the average of nine animals.
* The means of individual ratios found in the different animals.
These results were obtained on muscles in which the average gain in weight was 33 ± 3%. Similar results have also been obtained more recently when hypertrophy equalled 46 ± 3%.

TABLE II

Leucine-^{14}C Incorporation into Actomyosin during Hypertrophy

	Total fibrillar cpm	cpm extractable as actomyosin	Average of total extractable*
			%
Control soleus	1128 ± 129	802 ± 88	72 ± 2
Hypertrophied soleus	1711 ± 173	1228 ± 147	71 ± 3

Each value represents the average of five determinations, each of which was performed on the combined myofibrillar fractions of three muscles. All values are given ±S.E. of means. The hypertrophied muscles are the same ones studied in Table I.
* The mean of individual ratios found in different experiments.

TABLE III

Distribution of Leucine-^{14}C into Different Centrifugal Fractions

Fraction	Total sarcoplasmic cpm	
	Control soleus	Hypertrophied soleus
	%	%
Mitochondria (to 8,000 g)	7.8 ± 0.4	7.2 ± 2
Grana (to 30,000 g)	3.9 ± 0.3	4.5 ± 0.3
Microsomes (to 100,000 g)	4.9 ± 0.4	5.7 ± 0.4
Soluble	83 ± 1	83 ± 0.7
Total sarcoplasmic cpm	3297 ± 91	1931 ± 66

Each value represents the average of four different experiments, each of which involved the combined soluble fractions of three soleus muscles. The average per cent of total soluble radioactivity ± SE is given. Hypertrophy averaged 34 ± 3% in this experiment.

the label in the various fractions did not differ from that in the soleus.

DISCUSSION

Compensatory hypertrophy of skeletal muscle is accompanied by increased incorporation of labeled amino acids into proteins. Similar observations have been reported for cardiac muscle (6, 11, 13). Hypertrophy also increases such correlates of protein synthetic activity as the RNA content of the muscles (Torkelson and Goldberg. Data in preparation.) and the capacity of the muscle to concentrate amino acids from the blood (5).

By itself, however, the finding of greater incorporation of amino acids into protein does not necessarily indicate greater protein synthesis. Changes in the sizes, rates of labeling, or specific activities of intracellular amino acid pools can lead to greater incorporation of a radioactive pulse without any change in the rate of protein synthesis. Such complications appear especially important because the rate of entry of amino acids into muscle is influenced by the level of muscular work (5). These potential complications were minimized in the present chronic studies, in which total incorporation was measured after repeated subcutaneous injections of labeled leucine throughout the period of growth. Under these conditions, the increase in incorporation of labeled amino acids was directly related to the gain in muscle weight.

Although qualitative evidence for an association between growth and increased protein synthesis has been obtained in a wide variety of tissues, quantitative studies of this relationship have been reported only for exponentially growing bacteria (9). In such microorganisms, growth was also found to be linearly related to amino acid incorporation. Quantitative data, such as those presented in Fig. 1, are difficult to obtain in higher organisms because of technical problems in quantifying organ growth and the associated biochemical changes without using prohibitively large numbers of animals. In the present experiments this difficulty was circumvented by comparing data on paired growing and nongrowing muscles in the same hypophysectomized animal.

Interpretation of the linear relationship described in Fig. 1 is complicated by the phenomenon of protein turnover. In the absence of protein degradation, e.g. in exponentially growing bacteria (9, 10), the amount of growth must be proportional to the amount of new protein synthesized. This simple relationship cannot hold for muscle or other mammalian cells in which the half-lives of proteins are much shorter than the half-life of the cell. Even in the absence of growth, protein synthesis must occur in such cells to replace degraded proteins and to maintain cell size. Fig. 1 demonstrates that for muscle the amount of growth is proportional only to the amount of protein synthesized in excess of that required to balance degradative processes, i.e. in excess of the incorporation in the nongrowing control.

Since protein synthesis and protein degradation are equally balanced in nongrowing muscle, one can estimate, from measurements of average turnover rates of muscle protein, the amount of protein synthesized by the controls during the period studied. The smallest reported value for average half-life of skeletal muscle proteins is 23.4 ± 4 days (6). At this rate, 17 ± 2% of the original protein mass of the muscle would be catabolized in 6 days. The leucine-^{14}C incorporated into the nongrowing controls, therefore, corresponds to the replacement of this amount of protein. Doubling the incorporation of ^{14}C during hypertrophy would thus be expected to cause at most only a 17% gain in muscle weight, rather than the 40% gain

actually observed (Fig. 1). In this way, the actual increase in incorporation, i.e. the slope of Fig. 1, appears too small by a factor of 2 or 3 to account for the observed growth.

A number of possible effects could explain this result. (*a*) If the amino acid pool in the hypertrophying muscle had a lower specific activity than that in the control, the incorporation data would underestimate the protein synthesis in the growing muscle. Although information on amino acid pools in hypertrophying muscle is presently lacking, this possibility appears unlikely because hypertrophying muscles concentrate AIB-^{14}C from the blood more actively than controls (5). (*b*) Alternatively during hypertrophy, there could be increased synthesis of some muscle components, e.g. collagen, which contain little leucine and therefore would be poorly labeled. (*c*) The value used for the average half-life of muscle protein may be too large; thus a given amount of leucine incorporation could actually correspond to more protein synthesis than was estimated above. (*c*) It is also possible that decreased protein degradation is an important mechanism of compensatory growth. In fact, recent evidence indicates that average half-lives of muscle proteins are prolonged during hypertrophy (Goldberg. Data in preparation.). Further experiments are in progress to decide between these possibilities.

During hypertrophy, soluble and myofibrillar proteins appeared to be synthesized in approximately the same relative proportions as in nongrowing control (Table II). The data on the distribution of newly synthesized proteins into various sarcoplasmic fractions (mitochondria, Ca-binding grana, microsomes, and soluble proteins) argue against the disproportionate synthesis of any of these cellular components in response to increased work. Proportionate increases in the rates of synthesis of these various components, however, do not necessarily lead to proportionate increases in their concentrations in the muscle. Since the amount of any cellular component depends on both its synthetic and degradative rates (15), changes in protein half-lives during hypertrophy could alter the composition of the cell even though the rates of synthesis of these different components increase in parallel (Tables I and III). Thus, the present findings are not necessarily in disagreement with the earlier observation that the leg muscles of exercised rabbits have a higher content of myofibrillar proteins than those of restrained animals (8), or with the various morphological studies that have emphasized the increase in myofibrils during hypertrophy (1, 12). It is apparent that a full understanding of the cellular processes involved in compensatory growth will require information on rates of protein degradation as well as protein synthesis.

SUMMARY

The incorporation of leucine-^{14}C into proteins was studied during compensatory growth of the soleus and plantaris muscles. Growth of these muscles of one limb was induced by tenotomy of the synergistic gastrocnemius muscle. At the end of the growth period, these muscles showed greater incorporation of leucine-^{14}C into proteins than did contralateral controls, and the final gain in muscle weight was directly proportional to the increase in amino acid incorporation. Soluble and myofibrillar proteins appeared to be synthesized in the same proportions as in nongrowing muscles. The relative incorporation of leucine-^{14}C into various sarcoplasmic components (mitochondria, Ca-binding grana, microsomes, and soluble proteins) was also similar in hypertrophying and control muscles. The increase in amino acid incorporation, however, appears too small to account entirely for the gain in muscle weight.

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