# Myosin Heavy Chains in Striated Muscle Immobilized in a Shortened Position

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**Abstract**

In adult rat the total content of myosin heavy chain (MHC) and the MHC patterns were studied by biochemical methods in slow-twitch (soleus) and fast-twitch (gastrocnemius) muscles immobilized for 14 days in a shortened position. Ultrastructure of the contractile apparatus of the muscles was also examined.

After a 14-day immobilization the muscle mass is decreased by about 41% in the soleus and by about 25% in the gastrocnemius. Total MHC content decreases by about 70% and 40% in the slow and in the fast muscle, respectively. Ratios of MHC-1/MHC-2A(X) and of MHC-2A(X)/MHC-2B are diminished. Individual MHC isoform content decreases in both muscles: the MHC-1 by 60-70%, the MHC-2A(X) by 45-60% and the MHC-2B by 35%.

Electron microscopy analysis of the contractile apparatus shows both loss of individual myosin filaments and foci of missing myosin and actin myofilaments. Myosin to actin filament ratio is decreased, and accordingly, SDS-PAGE shows that the MHC to actin ratio was diminished.

The results show that following 14-day immobilization in a shortened position: (a) the slow muscle atrophies more than the fast muscle; (b) the content of MHC isoforms decreases, the loss of the MHC-1 being the largest, and that of the MHC-2B the smallest; (c) the myosin filaments disappeared before the actin filaments.

**Key words**: Actin filament, contractile apparatus, immobilized muscle, muscle ultrastructure, myosin filament, myosin heavy chains, striated muscle.

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It is well known that decreased activity causes muscle atrophy. Nevertheless, the type-dependent difference in the muscle response to inactivity is still not quite clear. Mechanism(s) by which disuse-atrophy develops is also far from being elucidated. Muscle reactions to inactivity and to "unloading" are particularly interesting while considering individuals spending long times in hypogravity. Since they undergo muscle weakness and atrophy.

Some unweighting-induced anomalies of the contractile structure were described as sarcomere lesions or focal loss of myofilaments [27, 28]. To mimic influence of hypogravity upon the muscle tissue, experimental models are applied on animals [8, 23, 28, 32, 33]. Muscle inactivity and some "unloading" can be obtained by immobilization of the muscle in a shortened position [17].

Velocity and power of muscle contraction also depend on the isoforms of myosin heavy chain (MHC), and is correlated with the muscle fibre type [7, 22, 30]. Change in muscle activity induces changes in the MHC isoform pattern, which marks the transformation of fibre types. Namely, increased, continuous, low frequency muscle activity causes transformation from the fast to slow type [4, 19, 21, 24], while long-term inactivity results in a shift from the slow to fast forms [23, 25, 26, 32]. However, there is some controversy concerning the cause of changes in the MHC pattern seen in inactive-atrophying muscle that is if only selective loss of particular isoform occurs, or if it is accompanied by an increase in other isoforms.

In long-term inactive muscle the fast-glycolytic fibres atrophy much less than the slow and the fast-oxidative fibres [23]. Short-term immobilization (4 days) results in some atrophy of the slow rat leg muscle, but it does not yet cause atrophy of the fast muscle, nor any changes in the MHC pattern, of both slow and fast muscles. However, when the muscles are immobilized in a shortened position, atrophy of both muscles and slow to fast shift in their MHC pattern are significant, as early as 4 days of experiment, though the content of the MHC-2B isoform is unaffected

[31]. In the present paper changes in the MHC isoforms of slow-twitch and the fast-twitch muscles are studied after immobilization in a shortened position for 14 days. The aim of the work was to explain how the contents of the MHC isoforms get changed in the inactive and somewhat "unloaded" muscles, and also to shed some light on the mechanism of atrophy in such muscles.

## Materials and Methods

*Animals and immobilization procedure*

Female albino Wistar rats (2.5-3 months old, 150-190 g) were used. They were fed a regular diet of laboratory chow and water ad libitum. The slow-twitch muscle, soleus (SOL) and fast-twitch muscle, gastrocnemius (GAST) of the same animals were studied. The muscles were immobilized in a shortened position, under ether anaesthesia, by immobilization of the ankle joint between aluminium roads at an angle of about 150°-160°. Muscles were examined after 4 and 14 days. As controls served: the muscles from the contralateral leg (Contralateral) and the muscles from nonexperimental animals from the same population (Controls). Muscles from 2-4 rats were used in each experiment.

*Preparation of myofibrils*

Animals were killed by decapitation after 14 days of muscle immobilization. The SOL and GAST muscles were immediately removed, measured weighed and transferred to ice. After removal of tendons and fasciae the muscles were homogenized. Crude myofibrils were separated as previously described in detail [13, 15]. Myofibrils from muscles of both experimental and contralateral legs were obtained under exactly the same conditions, carefully monitoring volume of solutions during the procedure; they were studied in parallel. The total content of protein in myofibrils was estimated by the method of Lowry et al. [20].

*Total content of MHC and actin*

The total contents of MHC and actin in the muscles were determined in crude myofibrils as previously described in detail [12, 14]. Myofibrillar proteins were separated by SDS/PAGE as described by Weber & Osborn [34]: samples (20-40 μg of protein) were applied to 6-8% acrylamide gels. After separation and staining with Coomassie Blue, individual bands were cut out and eluted from gels; the amount of protein was estimated colorimetrically. The intensity of protein staining was proportional up to 30 μg of protein within one band [12].

The amounts of the particular proteins in gels from the experimental muscle were compared with those from the control muscle. Gels from the same electrophoresis run, stained and destained together, were exclusively used for comparison. The data of each experiment were derived from the analysis of at least 8 electrophoretic runs.

*Analysis of MHC isoforms*

MHC isoforms were separated from isolated myofibrils by SDS/PAGE (6% gel) with high glycerol concentration (37%) as described by Carraro and Catani and modified by Danieli Betto et al. [2, 6]. Individual isoforms of MHC: 1, 2A, 2B and 2(X) were identified according to their electrophoretic mobility and immunochemical patterns, as quoted previously [12]. Under the electrophoretic conditions used, the MHC-2(X) and the MHC-2A isoforms have very similar mobility. Thus the MHC-2A band can also contain the MHC-2(X) isoform. In the soleus muscle this band contain exclusively MHC-2A, which is the only fast component of the rat soleus muscle myosin [3, 29]. Isoforms 2A and 2(X) [18] are also called 2d and 2a [1].

Relative amounts of MHC isoforms were determined by comparing the degree of intensity of staining with Coomassie Blue of the electrophoretic bands by using a Panasonic CCD Video Camera and digitized using IBM 386 computer equipped with Visionetics frame graber card as previously described [31]. A linear response for the individual MHC bands was obtained when 0.5 μg to 9 μg of myofibrillar protein was analysed. The data of each experiment derive from at least 8 independent electrophoretic runs.

*Preparation of samples for ultrastructural study*

Animals were killed by decapitation after 4 or 14 days of experiment. Immediately, each muscle was excised, maintaining its experimental length carefully, measured and attached to a plastic rod. Samples for study under the electron microscope were prepared as previously described [11, 15].

*Statistical analysis*

The results were evaluated statistically by the Student test and by the sign test [5].

## Results

*Muscle length and mass*

Length of the control muscle was 19.8 ± 0.2 mm and 23.3 ± 0.4 mm (mean values + SEM) for the SOL and GAST, respectively. Mass of the control muscles was 76 ± 1 mg and 973 ± 19 mg (mean ± SEM) for the soleus and GAST, respectively.

After immobilization in the shortened position the SOL decreased in length by about 15%, while the GAST of the same legs by 7%.

After immobilization in the shortened position the mass of the SOL muscles decreased by about 42%, while the mass of the GAST by about 25% (Fig. 1).

*Ultrastructural analysis*

The contractile structure of the control muscle was regularly arranged. Actin and myosin filaments formed a regular hexagonal array within the A-band (Fig. 2a).

In the 4-day immobilized muscles the length of sarcomeres was decreased and variable. In the 14-day immo-

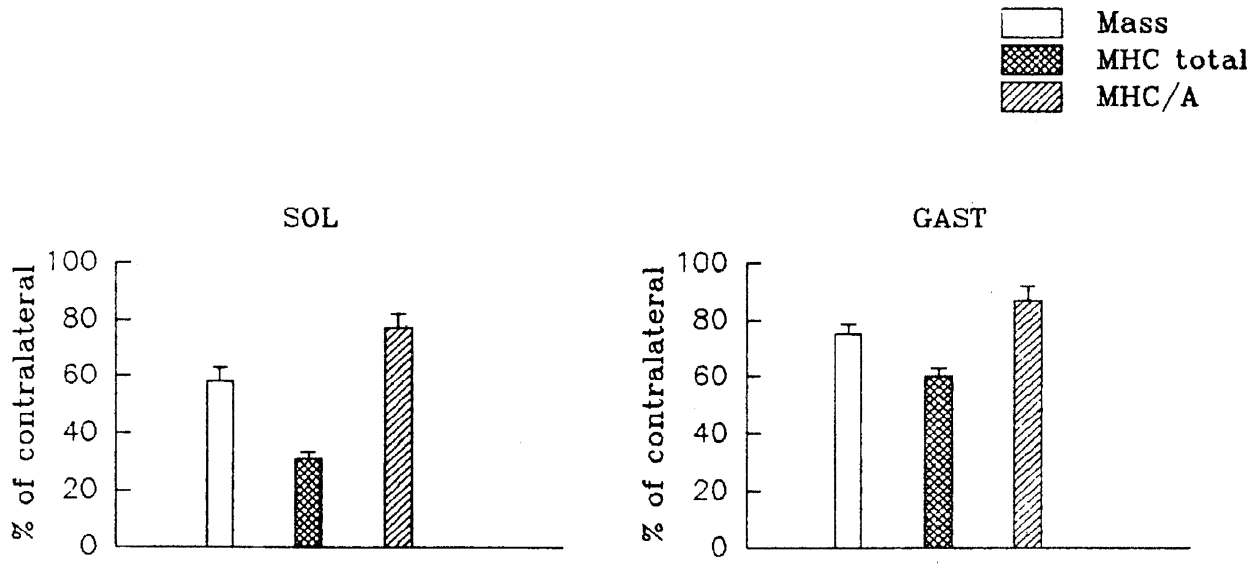


*Figure 1. Changes in muscle mass, total MHC content and in MHC to actin ratio in the muscles immobilized for 14 days in a shortened position. The muscle content of MHC and actin were evaluated as described in the Materials and Methods. Values are means ± SEM.*

bilized muscles the mean lengths of the sarcomeres were in the range of control, however, some variability in their length was still observed. Irregularities founded in the contractile structure of the immobilized-shortened muscles were similar to those described previously [11, 16, 28].

The lack of individual myosin filaments (Figs. 2b-d) and foci of missing myosin and actin myofilaments were present within the myofibrils showing regular structure (Fig 2b). These characteristic features were present in both the 4- and the 14-day immobilized-shortened SOL and GAST muscles. Increased proportion of the actin to myosin filaments was seen within the A-band in many areas: instead of the regular hexagonal array, more than 6 thin filaments surrounded one thick filament (Fig. 1b, c).

*Total MHC content and MHC to actin ratio*

After experimental day 14 the total content of MHC was decreased by about 70% i n the SOL and by about 40% in the GAST. The decrease in total MHC content was larger than that in muscle mass, the difference being more pronounced in the SOL than in the GAST (Fig. 1). The percentual decrease in the actin content was very similar to that in the muscle mass (data not shown).

MHC to actin ratio (MHC/A) was 1.95 ± .09 and 2.01 ± .08 in the control SOL and GAST, respectively. After 14 days of immobilization in shortening the MHC/A ratio decreased in both experimental muscles: by about 23% in SOL muscle and by about 13% in GAST (Fig. 1).

*MHC isoforms*

The MHC of the control SOL contained about 77% of type 1 isoform and about 23% of type 2A. The MHC-2B isoform was not detected in gel s of the SOL muscles in the present experiments.

The MHC of the control GAST muscle contained about 5% of type 1 isoform, about 38% of 2A(X) and about 57% of 2B isoform.

In the SOL muscle immobilized for 14 days in a shortened position, proportion of MHC-l decreased, whereas MHC-2A increased by about 4% (Table 1). In the 14-days immobilized GAST proportion of the MHC-1 and MHC-2A(X) decreased by about 1% and 3%, respectively, whereas the proportion of the MHC-2B increased by about 4% (Table 1). Thus, in both muscles the shift in proportion of MHCs from slow-to-fast isoform took place.

To establish changes in the real amount of the individual MHCs in the experimental muscles, the data presented in Table 1 and Fig. 1 were recalculated, and content of the individual MHC isoforms was expressed relatively to 100 mg of the total MHC content (Table 2). Such analysis shows that all the MHC isoform decreased in the immobilized muscles. The loss of the MHC-l was the largest, while that of the MHC-2B was the smallest. Losses of the corresponding isoforms were larger in the SOL than in GAST (Table 2 and Fig.3).

## Discussion

Atrophy of the muscle immobilized in a shortened position comes as a result of at least two different factors, namely muscle inactivity and muscle shortening. The shortening causes a muscle "unloading", which is a factor that decreases function of the afferent system, and in consequence, it lowers the muscle activity and causes muscle atrophy [8, 17]. The slow muscle is known to be more sensitive than the fast muscle to changes in activity of the afferent system. The shortened position is the additional cause of muscle atrophy, bringing about elimination of part of the contractile structure [11, 35]; this phenomenon

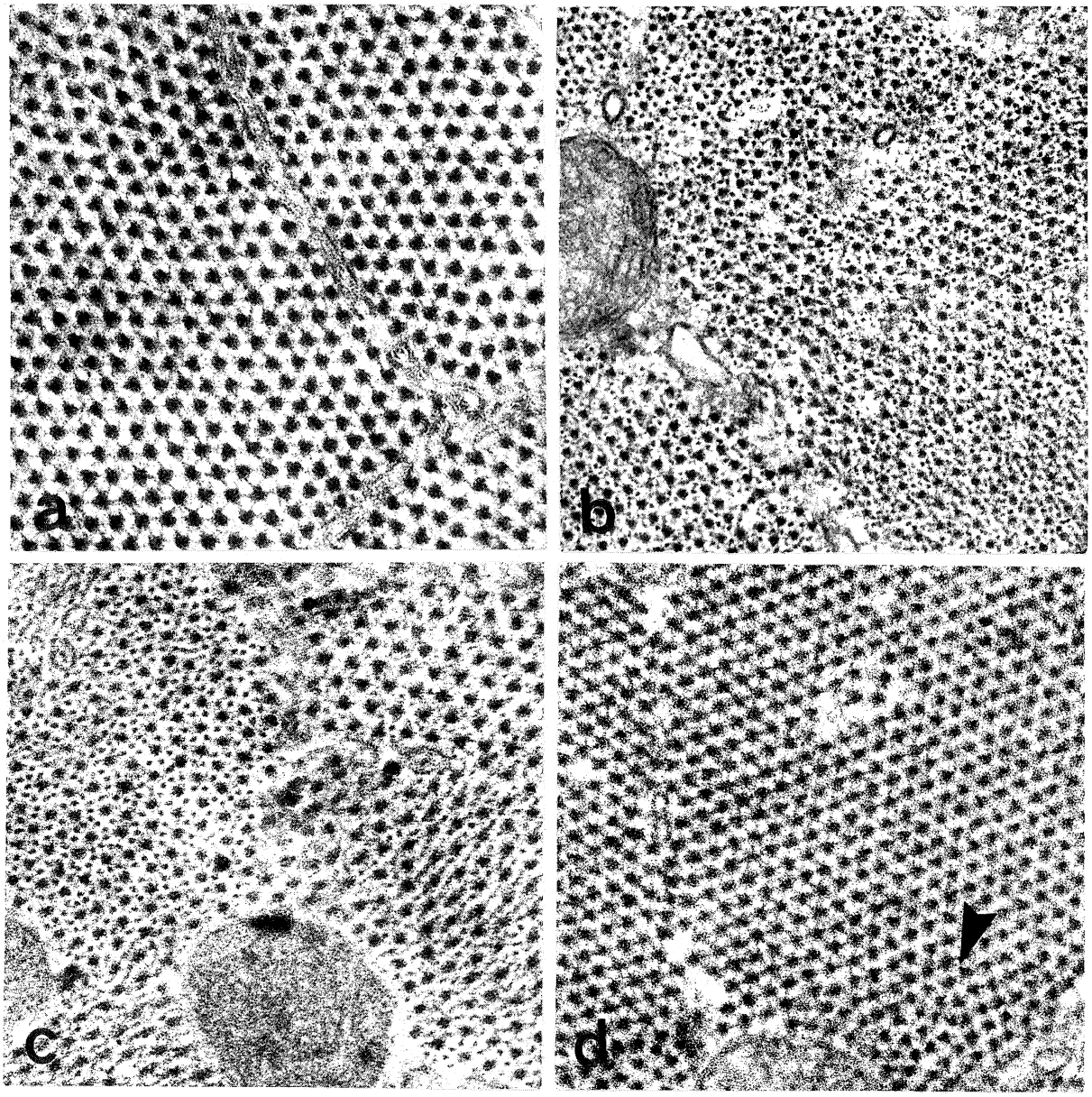


*Figure 2. Electron micrographs of the studied muscles. Transverse sections. Control muscle (a); Muscles immobilized in a shortened position: SOL for 4 days (b), SOL for 14 days (c), GAST for 14 days (d). Foci of missing myofilaments (b-d). Loss of hexagonal arrangement of filaments and excess of actin filaments (c, left-hand side). Loss of hexagonal arrangement of filaments (d, arrow). (a) x 110000; (b) x 90000; (c) x 115000; (d) x 90000.*

seems to be independent of the type of muscle. The shortened muscle resembles the tenotomized muscle in many aspects [17].

It is well known that long-lasting muscle inactivity causes disuse atrophy in the muscle of every type, but the fast-glycolytic fibres are less affected by inactivity than the fast-oxidative and the slow fibres [23]. The early response to inactivity is also quite different in the slow and the fast muscles. The slow muscle atrophied soon, whereas the fast muscle seems to be "resistant" to short-term inactivity [31]. The atrophy caused by the muscle immobilization in a shortened position was also larger in the SOL than in the GAST of the same leg (Fig. 1). This phenomenon could be explained in part by greater shortening of the SOL muscle than of the GAST one, perhaps due to anatomical insertions (discussed in [12, 31]). Yet, after 14-day immobilization in a neutral position, atrophy of the SOL muscle was larger than that of the GAST of the same leg (results not shown). Thus, the short-term immobilization causes atrophy in both muscles, but it is more evident and begins earlier in the slow than in the fast muscle.

The present changes in the pattern of MHC isoforms are, in general, similar to those previously found in several models of disuse-atrophy [25, 26, 32]. They differ from the

*Table 1. Changes in the MHC pattern in the immobilized-shortened muscles. The MHC isoforms were electrophoretically separated; electrophoretograms were scanned and the relative amounts of individual MHC isoforms were evaluated as described in the Materials and Methods. The sum of all MHCs in the sample was taken as 100%. Results from several independent estimates were pooled. Values are means ± S.E.M. For statistical evaluation by the sign test [5], the amount of individual MHC isoform in the sample from the experimental muscle was compared with the corresponding one from the contralateral muscle, running in the same electrophoretic run. At least 12 such pairs from each group were evaluated.*

| Muscle | | Amount of the MHC isoforms | | |
|---|---|---|---|---|
| | | 1 | 2A(X) | 2B |
| SOL | Contralat. | 77 ± 2.0 | 23 ± 2.0 | |
| | Exp. | 72 ± 1.5 *** | 28 ± 1.5 *** | |
| GAST | Contralat. | 5 ± 0.3 | 38 ± 0.8 | 57 ± 0.1 |
| | Exp. | 4 ± 0.4 ** | 35 ± 0.6 *** | 61 ± 0.5 |

Differences statistically significant:
** $p < 0.01$; *** $p < 0.001$.

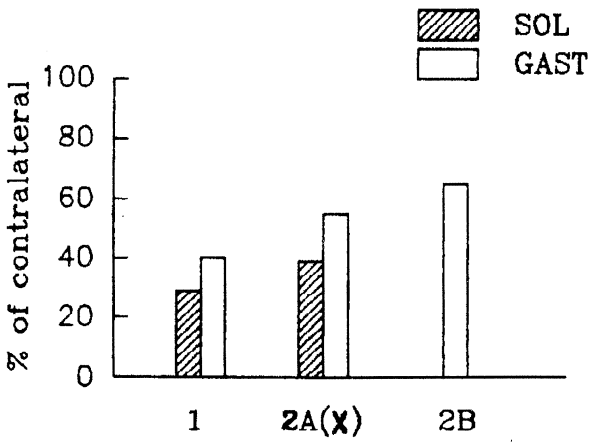


*Figure 3. Changes in content of the particular MHC isoforms in the 14-day immobilized-shortened muscles. Content of the MHC isoform of the experimental muscles, recalculated as in the Table 2, expressed as percent of the contralateral value.*

MHC changes in the denervated muscle [13]. In the rat leg muscles the shift in the MHC pattern is similar in immobilization in a shortened position for 14 days (Table 1), for 4-days [31], and in the tenotomy [12]: the proportion of the MHC-1 decreases, the proportion of the MHC-2B increases, whereas the changes in proportion of the MHC-2A(X) were muscle dependent. In all the above mentioned muscles evaluation of the amount of the individual MHC isoforms show some loss in all of them (Table 2, Fig.3, [12, 31]). However, the decrease in the content of the particular MHC isoforms was various, depending on the muscle type and on kind of experiment. In each of those experimental muscles the loss of the MHC-1 was the largest, while the loss of the MHC-2B was the smallest. Thus, all the MHC-isoforms of the rat leg muscles under study are sensitive to muscle inactivity, but to a different degree. Their sensitivity can be expressed as follows: MHC-1 > MHC-2A(X) > MHC-2B.

In ultrastructure of the contractile apparatus the selective loss of the individual myosin filaments and the diminution in proportion of the myosin to actin filaments were seen (Fig. 2). The changes were confirmed by the decrease in the total MHC content and by the diminution of the MHC to actin ratio estimated by the biochemical method (Figs. 1,3). Thus, it can be concluded that in the inactive-atrophying muscle loss of myosin filaments precedes loss of actin filaments. We observed the same phenomenon in denervated muscles [10, 13, 14, 15]. It seems that, such sequence of loss of filaments is a more general rule in the process of muscle atrophy. The difference between the immobilized and the denervated muscles seems to consist mainly in the

*Table 2. Content of the particular MHC isoforms in the immobilized-shortened muscles. Data presented in Fig.1 concerning amount of the total MHC and data presented in Table 1 concerning proportion of the MHC isoforms were recalculated and expressed relative to 100 mg of the total MHC muscle content.*

| Muscle | | HC-Total | | MHC-1 | | MHC-2A(X) | | MHC-2B | |
|---|---|---|---|---|---|---|---|---|---|
| | | Content (mg) | Loss (%) | Content (mg) | Loss (%) | Content (mg) | Loss (%) | Content (mg) | Loss (%) |
| SOL | Contralat. | 100 | | 77 | | 23 | | | |
| | Exp. | 31 | 69 | 22 | 71 | 9 | 61 | | |
| GAST | Contralat. | 100 | | 5 | | 38 | | 57 | |
| | Exp. | 60 | 40 | 2 | 60 | 21 | 45 | 37 | 35 |

larger disproportion of myosin to actin filaments in the denervated muscle than in the immobilized one (in spite of similar degree of atrophy in both muscles). Before long it leads to a disturbance in the hexagonal arrangement of filaments in the denervated muscle [14, 15]. On the other hand, in the immobilized muscle both the thick and thin myofilaments disappear more focally, while the structure of surrounding myofibrils is kept regular much longer (Fig.2 and [27]). Those differences are more evident in the slow than in the fast muscle.

In the inactive muscle, atrophy advances and the content of myosin heavy chains decreases (Figs. 1,3). It means that, processes of protein degradation prevail upon those of the protein synthesis. On the other hand, the transformation of the fibre types is a result of increased selective degradation of certain proteins and, simultaneously of selectively increased synthesis of some proteins [25, 32], in spite of diminution of the total synthesis of muscle proteins [9]. All that suggests that in the inactive-atrophying muscle both synthesis and degradation of individual proteins are selectively regulated.

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