

# Relationship between muscle myosin isoforms and contractile features in rabbit fast-twitch denervated muscle

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**Abstract** The effects of 8-day-old rabbit fast-twitch gastrocnemius denervation on the type of myosin isoforms and on contractile features (maximum velocity  $V_{\max}$  and contraction time (CT) of the muscle were followed between 15 and 60 days postnatal. The myosin isoforms and the  $V_{\max}$  and CT values of the denervated gastrocnemius displayed large changes during this period. These changes, which led at 2 months postnatal to a muscle displaying the properties of a slow-twitch muscle did not occur in synchrony: complete conversion to slow-type myosin isoforms occurred only at 60 days postnatal, whereas complete conversion to slow-twitch  $V_{\max}$  and CT values occurred as soon as 35 days postnatal. The results address a new question concerning the relationship between muscle myosin and contractile features.

**Key words:** Myosin isoforms; Muscle contraction; Muscle denervation; Rabbit

## 1. Introduction

In 1967, Barany [1] established a fundamental relationship between the speed of muscle shortening and the ATPase activity of myosin: adult skeletal muscles, which contain slow-type myosins having a low ATPase activity, display a lower speed of shortening than muscles, which contain fast-type myosins having a high ATPase activity. In the present work, we show that in the denervated gastrocnemius the relationship is not always holding.

## 2. Materials and methods

The right gastrocnemius of 8-day-old rabbits (New-Zealand) was denervated as described earlier [2]. When the rabbits were between 15- and 60 day-old, the denervated muscles (which showed no sign of reinnervation) and the contralateral muscles were removed. Myosin preparation, electrophoresis of native myosin isoforms, and measurement of contractile features (maximum velocity  $V_{\max}$  and contraction time (CT) were done according to procedures outlined previously [2].

## 3. Results

Rabbit gastrocnemius medialis is a fast-twitch muscle, which contains about 80% fast-type and 20% slow-type myosins [3]. The proportion of the slow-type was reached at about 6-week postnatal, after a small increase between birth and this age

(Fig. 1). During the same period, the  $V_{\max}$  of the muscle increased from a value of about 2 Lo/s to less than 3 (Fig. 2A).

The gastrocnemius, which had been denervated at 8-day postnatal, exhibited different features. We observed a large increase with age in the relative proportion of the slow-type myosins, which reached almost 100% at 2 months (Fig. 1). The age at which its value attained 50% was 35 days postnatal. During the same period, the  $V_{\max}$  of the denervated gastrocnemius decreased from a value of about 2 Lo/s to about 0.5 (Fig. 2A). This low  $V_{\max}$  value was reached at 35 days postnatal and was equal to the value obtained at this age for a rabbit slow-twitch muscle, the soleus (not shown). The twitch observed at this age was also that of the soleus (CT = 150 ms), three times larger than that of the contralateral gastrocnemius (CT = 50 ms) (Fig. 2B).

## 4. Discussion

In the control gastrocnemius, between 8 and 60 days, the  $V_{\max}$  displayed a small increase, while the relative proportion of the slow-type myosins also smoothly increased. This apparent contradiction may be explained by the fact that, during this period, the so-called perinatal myosins totally disappeared;

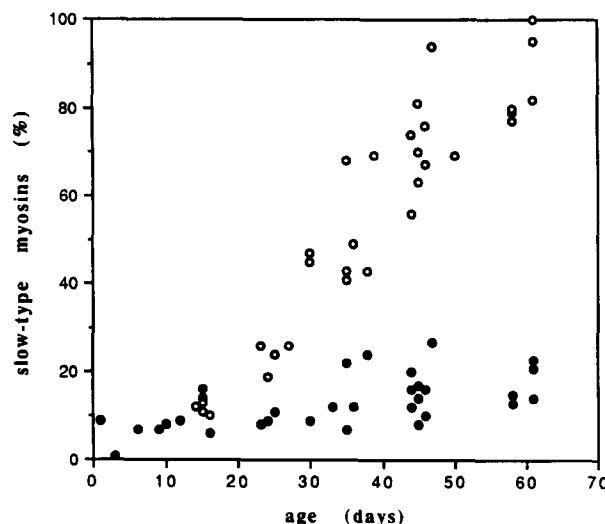


Fig. 1. Changes with age of the relative proportions (with respect to total myosin) of slow-type myosins in contralateral (●) and denervated (○) gastrocnemius. Before 15 days, the filled symbols correspond to control muscles. The proportions of slow-type myosins were determined by electrophoresis of native myosin isoforms under non dissociating conditions, in the presence of sodium pyrophosphate.

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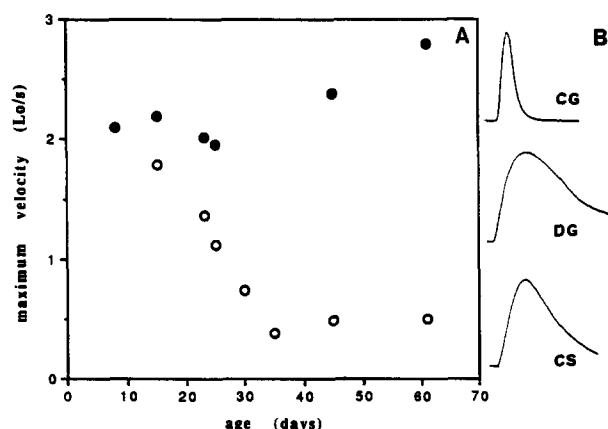


Fig. 2. (A) Changes with age of the maximal shortening velocity  $V_{max}$  of contralateral (●) and denervated (○) gastrocnemius. The point at 8 days postnatal corresponds to control muscles. (B) Twitch at 35 days postnatal of contralateral (CG) and denervated (DG) gastrocnemius and of control soleus (CS).

now, the gastrocnemius of a four-day-old rabbit, containing only this type of myosin, displayed a  $V_{max}$  value of 2 Lo/s (not shown). The increase in the Lo/s values determined between 8 and 60 days postnatal were thus the result of the progressive replacement of the perinatal-type myosins, contained in a muscle with a lower  $V_{max}$  (2 Lo/s), by the fast-type myosins, contained in a muscle with a higher  $V_{max}$  (3 Lo/s), reflecting the lower actin-activated ATPase of rabbit perinatal- relative to fast-type myosins [4].

In the 8-day-old denervated gastrocnemius, there was both a large increase in the relative proportion of the slow-type myosins and a large decrease in the  $V_{max}$  between 15 and 60 days. However, the two changes did not follow the same age dependence, since the minimum value for  $V_{max}$  was reached at 35 days postnatal, at an age where the relative proportion of slow-type myosins was still only 50%.

The asynchrony in a muscle between the changes in the  $V_{max}$  values (and also the CT values, not shown) and the content in slow-type myosins was shown here, as far as we know, for the first time. The results address a new question concerning the

relationship between muscle myosin isoforms and contractile features. One may propose at this stage two reasons for the unexpected observed asynchrony.

First, the cytochemical (myofibrillar ATPase) and immunocytochemical (myosin-isoform-specific antibodies) muscle sections of 60-day-old rabbit denervated gastrocnemius have shown in a previous work [2] that the slow-type fibers of this muscle are hypertrophied, while the fast-type fibers are atrophied. This was also observed in the present work at 35 days. In addition to other mechanical changes due to denervation, such as an increase in collagen, it is thus possible that the slow-type hypertrophic fibers predominated mechanically over the numerous small fast-type fibers and thus conferred to the denervated gastrocnemius, already at 35 days, the  $V_{max}$  and the CT values of a slow-twitch muscle.

Second, it has been shown by Zorzato et al. [5] that the denervation of adult rabbit gastrocnemius alters the functional properties of the  $Ca^{2+}$  release channels and Leeuw et al. [6] have reported that denervation of rabbit fast-twitch muscle enhances the production of slow troponin subunit isoforms. This suggests that, in addition to myosin, other proteins contribute to the mechanical properties of the denervated gastrocnemius.

To better apprehend the origin of the lag observed between the age variations of slow-type myosin relative proportions and  $V_{max}$  (and CT) values, the time-course of the production in the rabbit denervated gastrocnemius of proteins implicated in the calcium regulatory mechanisms is currently under study.

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