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Myosin gene expression in the respiratory muscles

J.G. Gea

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ABSTRACT: Myosin is one of the basic structural components of skeletal muscles. Its interaction with actin results in muscle contraction. The myosin molecule is composed of two heavy (MyHC) and two light chains (MyLC) that, together with the adenosine triphosphatase (ATPase) activity, determine the functional characteristics of the fibre. Both MyHC and MyLC present different isoforms. The main MyHC isoforms in adult mammals are the slow MyHC (MyHC-I) and fast MyHCs (MyHC-IIa, MyHC-IIb and MyHC-IIx). Muscle fibres can express only one isoform or coexpress different forms.

The muscle phenotype is the product of genome plus environmental stimuli. The family of genes that codifies the MyHC isoforms is located in two different clusters, each isoform being encoded by a separate gene. The gene corresponding to slow MyHC is located in chromosome 14, both in humans and in mice. The other genes are positioned in chromosome 17 in humans, and in chromosome 11 in mice. The transcriptional and translational mechanisms that control the expression of MyHC isoforms are not well known, although it is believed that the main regulation is dependent on mechanical signals. These signals are probably mediated by a biochemical messenger. As a general rule, fast MyHC genes seem to be expressed "by default", whereas the slow MyHC gene would be expressed as a response to changes in load.

So far, few studies have analysed the *in vivo* regulation of MyHC gene expression in respiratory muscles. It has recently been reported that breathing against moderate levels of inspiratory resistance quickly induces an increase in the genetic expression of slow MyHC in the diaphragm. This suggests the possibility of eliciting a phenotypic adaptation of respiratory muscles using specific training protocols.
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Correspondence: J.G. Gea
Grup de Pneumologia
Respiratory & Environmental Research Unit
IMIM-H. Mar
c/ Dr Aiguader 80
E-08003 Barcelona
Spain

Muscles are extremely plastic, and are capable of modifying their structure to the activity they develop [1–4]. At the molecular level, muscles are composed of different structural and enzymatic elements, whose genetic expression is modulated by such activity. Myosin is one of the main structural components of the skeletal muscles, which include respiratory muscles. This myofibrillar protein (fig. 1), essential for muscle contraction, is composed of two heavy (MyHC) and two light chains (MyLC). The MyHC (molecular weight 220 kDa) has a globular portion at one of its ends (amino-terminal part). This site is called the "head" (or S_1) of the molecule, and is the site of interaction with actin. This is also the site of action for the actomyosin adenosine triphosphatase (ATPase). The two MyLC (molecular weights, 17–23 kDa) are located very close to this portion, in the "neck" of the molecule. On the other side, there is an alpha-helicoidal structure (carboxy-terminal part) called the "tail". Both MyHC and MyLC present different isoforms. The presence of one or another isoform conditions the acti-vity of the myosinic ATPase [5], and these two factors determine the maximum velocity of shortening, the predominant metabolism of the fibre and its resistance to fatigue [1, 6–10]. In this regard, those fibres containing slow MyHC isoforms present a smaller contraction velo-city but higher metabolic efficiency for maintaining similar levels of tension [11]. The relationship between the MyHC composition and fibrillar function has been evidenced, not only in skeletal limb muscles but also in the diaphragm [12]. The MyLC isoforms are involved in force transduction, and, thus, in the mechanical efficiency and economy of different kinds of contraction [13].
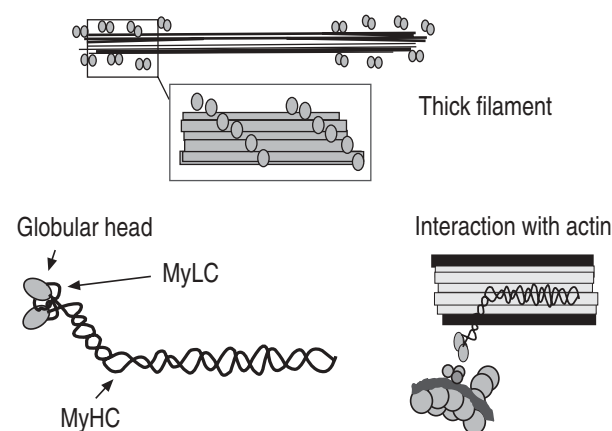

Fig. 1. – Molecular structure of myosin. MyLC: myosin light chain; MyHC: myosin heavy chain.

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In the skeletal muscles of adult mammals, four main types of MyHC have been described: one slow (slow-MyHC, MyHC-I or beta-MyHC) and three fast (MyHC-IIa, MyHC-IIb and MyHC-IIx) [14, 15]. In the early stages of development, foetal as well as neonatal MyHC isoforms exist. On the other hand, there are three main MyLC isoforms, MyLC1s, MyLC1f and MyLC3f. It is likely that these classifications still remain incomplete, above all regarding slow isoforms [14, 15]. The structure of different MyHC isoforms is similar among different species, and their velocity of contraction is specific for each isoform [1, 6, 7, 12, 16, 17]. Fibres containing MyHC-IIa have a velocity of shortening very close to those containing MyHC-IIx, and lower than those fibres containing MyHC-IIb [7, 12, 17]. The four main MyHC isoforms determine the four types of fibres (I, IIa, IIb and IIx) [18], although there are also mixed fibres, with different combinations of MyHC isoforms [18–23]. Generally, mixed fibres showed MyHC isoforms with similar functional and biochemical characteristics (*i.e.* MyHC-IIa and MyHC-IIx). However, it is also possible to detect fibres containing MyHC with very "incongruous" properties (*i.e.* MyHC-I in fast fibres). In human beings, fibres type I and type IIa are composed mainly of their corresponding isoforms (MyHC-I and MyHC-IIa, respectively). However, and interestingly, type IIb fibres seem to be predominantly formed by an isoform equivalent to the MyHC-IIx of rodents [18]. This is important for the respiratory muscles, since the diaphragm has a high proportion of this MyHC in many species [24]. The presence of MyHC-IIx and the presence of abundant oxidative enzymes [25], provide the respiratory muscle fibres with an important level of resistance to fatigue [26].

The expression of MyHC isoforms in the diaphragm varies from one species to another. In humans, slow and fast MyHC isoforms are expressed in similar amounts [27]. The same occurs in rats [28–30]. Interestingly, there is almost no MyHC-IIb in the latter animal [30]. By contrast, mongrel dogs show a higher expression of the fast MyHC isoform in this muscle [31]. The expression appears as homogeneous throughout the diaphragm, with no differences between the costal and the crural portions [31].

As mentioned above, the skeletal muscle has a great capacity for structural adaptation. The phenotypic characteristics of a muscle are determined by genes and the influences of the environment on their expression [32]. In recent years, techniques in molecular biology have made it possible to identify the genes encoding the different MyHC and MyLC isoforms [33]. These genes form three multigenic families, each one probably being derived from a common gene, called the "ancestor gene". The superfamily of genes encoding MyHCs is composed of different genes, each one appearing to encode one specific isoform. The MyHC genes are located in two clusters (fig. 2). The one for slow MyHC is located in chromosome 14, closely linked to that of cardiac alpha-MyHC, both in humans and mice [34–36]. The other MyHC genes, including those encoding fast MyHC isoforms, but also foetal and perinatal MyHC, are clustered in chromosome 17 in humans, and in chromosome 11 in mice [36, 37]. At least six genes exist in this cluster [38, 39], and it is believed that there is a specific
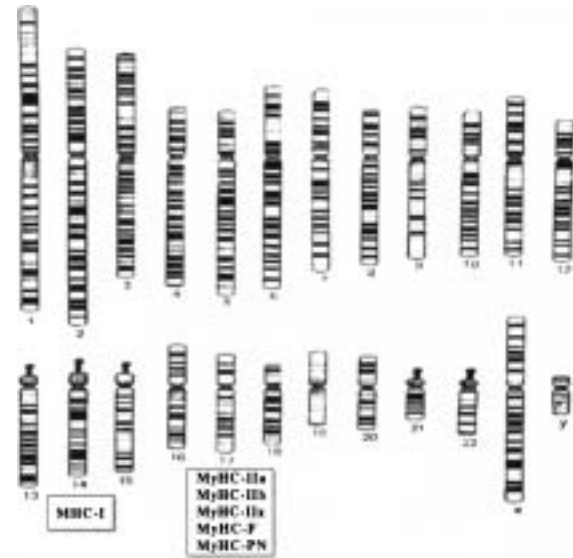

Fig. 2. – In humans, the genes for slow myosin heavy chain (MyHC-I) are clustered in chromosome 14, whereas those for fast, foetal and perinatal myosin heavy chain (MyHC-IIa, b and x, -F and -PN, respectively) are located in chromosome 17.

gene for each one of the fast isoforms (MyHC-IIa, MyHC-IIb and MyHC-IIx) [33].

Genes are transcribed into messenger ribonucleic acids (mRNAs), that are specific for each isoform. Studies using *in situ* hybridization (ISH) have made it possible to identify these mRNAs. This has improved knowledge of the next steps necessary for the synthesis of MyHC molecules and their assembly in the thick filament of the sarcomere [40–42]. The mRNAs corresponding to MyHC have been detected predominantly in the periphery of the fibres, surrounding the nuclei [43] (fig. 3). Smaller concentrations have been observed close to the A-band of the sarcomere. This suggests both that the message and not the product (MyHC) emigrate to the incorporation site, and that the MyHC translation ends up in the interfibrillar space, in the periphery of the myofibril [44–46]. There, the new MyHC diffuses and is incorporated throughout the width and length of the A-band [46]. The studies using ISH have also confirmed the heterogeneity of many fibres, that simultaneously co-express genes encoding different MyHC isoforms [33].
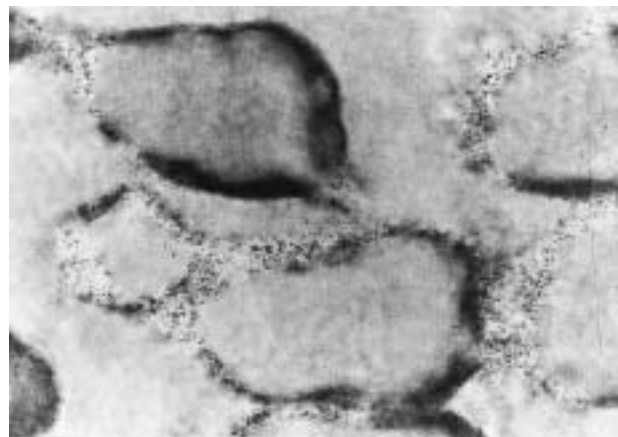

Fig. 3. – Expression of messenger ribonucleic acid (mRNA) corresponding to slow myosin heavy chain observed in a cross-sectional view of fibres from the diaphragm.

The transcriptional and translational mechanisms that regulate the expression of the genes encoding MyHC isoforms are not well-known, although they are being actively investigated. It is believed that the expression is regulated fundamentally by mechanical signals, particularly by changes in the tension generated by the muscle during the effort [47]. The stretch (active tension) as well as electrical stimulation (passive tension) provoke the repression of the fast-type genes, with activation of the slow-type genes [2, 48]. This implies a progressive transformation in the type of fibres [49–52]. This transformation is not dependent on their substitution by new fibres, but on changes in the molecular expression of their MyHC isoforms [53]. As a general rule, the fast-type genes seem to be expressed "by default", whereas the slow-type genes would be expressed as a response to changes in loads [50–52]. Thus, a decrease in or lack of activity would result in a higher expression of fast MyHC, with an increase in the size and/or proportion of type I fibres [54–58]. However, the type of activity also influences the expression [59]. For example, aerobic exercises trigger the expression of slow MyHC, whereas weight-lifting training induces a greater expression of fast MyHC [59]. Rest and weight-lifting exercises act in different ways, increasing the expression of fast MyHC. Whereas the latter provoke an increase in expression of fast MyHC in fast fibres that become hypertrophic, the decrease in activity results in a higher expression of fast MyHC in the overall population of fibres. Apparently, transitions among different types of MyHC seem to follow an order established by their functional characteristics. Thus, MyHC-I would be substituted by MyHC-IIa, which in turn would be sequentially replaced by MyHC-IIx and MyHC-IIb [60, 61]. This sequence would operate in both directions, according to the type of stimulus experienced.

As mentioned previously, mechanical signals are believed to be the main stimulus capable of inducing changes in the expression of MyHC isoforms. The effects of active tension have been studied, submitting the muscle either to an increase or decrease in loads [54, 62–64]. For the former, muscle overloading [50, 64, 65] and training [59, 66–71] were used. In contrast, suspension [50, 54, 57, 65, 67, 72], immobilization [2, 72] and microgravity [56, 73] were employed to reduce the loads. On the other hand, passive tension has been evaluated through studies using different patterns of neural activity [1, 51, 74–77]: electrical stimulation [51, 53, 77–81]; muscle blockade [83–85]; and denervation [82]. Finally, hormonal factors, such as the effects of thyroid hormone or steroids, also influence the expression of different MyHC isoforms [66, 86–88]. The above-mentioned factors were used both separately and in a combined form to evaluate the specific weight of each one [65, 67]

Nutrition is an additional factor that can modulate the genetic expression of MyHC. Some dietary deficiencies during development [89], and local ischaemia [90], appear to favour the expression of slow MyHC. Sex, degree of development and ageing are other factors that condition the expression of MyHC [91]. At the moment of birth, skeletal muscles show a combination of embryonic and neonatal MyHC, with small quantities of MyHC-I and MyHC-IIa. The embryonic MyHC is progressively substituted by adult forms [92, 93]. Ageing involves new changes in expression. As an example, MyHC-IIb is substituted by MyHC-IIx in older rats [94].

A point of interest is the intensity of the stimulus capable of triggering changes in the genetic expression of MyHC. Although even moderate loads are capable of modifying this expression [31], the minimum threshold required to induce the transformation still remains unknown. In general, the intensity and velocity of the changes vary from one animal species to another [95]. A factor that should also be considered is the duration of the stimulus required for transformation [96]. In adult animals, changes have been observed even after extremely short periods of stimulation [48].

It is believed that changes in the tension generated by the muscle during the effort would be the initial phenomenon involved in the transformation process [47]. A fascinating question is how changes in tension result in changes in MyHC gene expression. The signal could act just mechanically or through the release of a messenger. However, recent studies suggest that the muscular activity itself is not sufficient for triggering the process [47]. Nevertheless, the link between the mechanical signal and changes in MyHC gene expression remains undiscovered. It is possible that some of the mediators that act in the early stages of development, such as myogenin and/or MyoD, could be implicated [97]. The persistent decrease in the potential of phosphorylation in the adenosine triphosphate (ATP) system has also been suggested as a potential trigger for MyHC transformation [95]. In addition, a growth factor that has recently been cloned from muscles subjected to stress can also be implicated in this genetic modulation [98]. On the other hand, membrane and sarcomere damage have been observed in muscles after moderate loading [99]. This damage coexists with changes in the genetic expression of MyHC [31], and with the presence of the above-mentioned growth factor [98]. The relationship between muscle damage and changes in MyHC expression is also unknown. The first event may be a prerequisite for gene switching or just a different part of the same remodelling process, not directly related to the latter.

The capacity of muscles to adapt to a variety of challenges has been explored mainly in the field of sports medicine, and in the treatment of cardiac diseases with myocardial substitutes [100, 101]. There are few studies evaluating these phenomena in respiratory muscles. In general, the results are quite similar to those observed in other skeletal muscles submitted to loads [83, 101, 102]. Interestingly, general training, although prolonged, appears to result in only mild changes in the cellular and molecular characteristics of the diaphragm [103]. In addition, changes appear to affect just the costal portion of the diaphragm. In contrast, we have recently observed that specific respiratory stimuli are capable of dramatically modifying the structure of this muscle *in vivo*. Breathing against moderate levels of inspiratory resistance resulted in an increase in the expression of the genes encoding the slow MyHC [31]. These changes affected both the costal and crural portions of the diaphragm. The transient submission to respiratory loads probably emulated an endurance training, with similar results in MyHC expression [70]. The nature of these
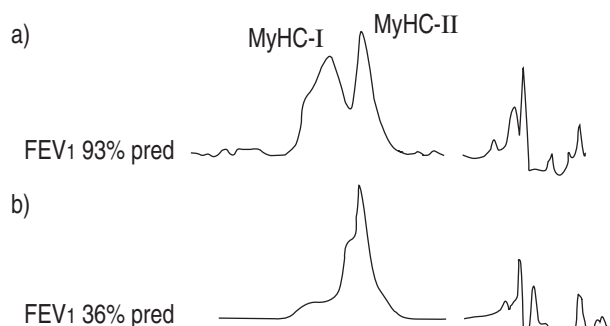
Fig. 4. – Densitometric profiles of slow (MyHC-I) and fast myosin (MyHC-II) heavy chain isoforms obtained in electrophoresis from the external intercostal muscles of subjects with: a) normal lung function; and b) severe chronic obstructive pulmonary disease (COPD). FEV_1: forced expiratory volume in one second; % pred: percentage of predicted value.

changes is presumably adaptive. Thus, the phenotypic reprogramming of the muscle with a greater expression of genes encoding slow MyHC would condition multiple metabolic and mechanical advantages. The smaller energy cost necessary to maintain a similar tension [5, 11, 104], would be added to a higher resistance to fatigue [70]. However, not everything is beneficial; these changes would also result in a higher curvature of the force-velocity relationship in the muscle, with loss both of velocity and strength of contraction [7, 8, 17]. On the other hand, it has recently been observed that external intercostal muscles show an increased expression of fast MyHC in patients with chronic obstructive pulmonary disease (COPD) [105] (fig. 4). This increase, in the opposite direction to what one would expect in the diaphragm, is probably related to the type of activity that external intercostals have to develop in COPD patients. On the other hand, our group was not able to demonstrate changes in the expression of MyHC in the diaphragm of these patients [27]. However, this could have been due to the fact that patients with severe airways obstruction were not included.

An interesting implication of these studies is that they suggest the possibility of eliciting a phenotypic adaptation of the diaphragm in humans using training programmes. The beneficiaries of such programmes would be mainly COPD patients, but also those individuals submitted to long-term mechanical ventilation. The programmes should be designed to achieve specific objectives. Improving the strength of respiratory muscles can be useful for some functions (*i.e.* cough and sighs in exacerbations of chronic respiratory diseases), whereas increasing the endurance would be useful in others (*i.e.* exercise tolerance). Possibilities other than training could probably be contemplated in the near future, such as the use of drugs known to modify the genetic expression of structural proteins, or the use of genetic therapies to induce the synthesis of endogenous growth factors in the muscle.

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