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Muscle Fiber Types and Sport Medicine: an update in 2005

Abstract

It has been known for a long time that skeletal muscle tissue is a heterogeneous organ in term of function and correlated architecture. Physiological and biochemical research allowed developing numerous classification schemes based on metabolic and ultrastructural characteristics to describe muscle fibers. These delineation methods have been shown to be correlated between them or not depending on the cases. Furthermore, recent research has shown that muscle fiber type transitions in response to environmental changes encompass a growing number of proteins, either in a coordinated or uncoordinated form. This yields numerous «atypical» muscle fiber types possessing mixed characteristics previously attributed to distinct fibers, creating a continuum in analyzed samples. Stimuli driving muscle fibers to switch their phenotype have also been addressed, allowing to more or less predict the type and extent of transitions obtained using standardized protocols on a set of defined muscle groups.
At the same time, comprehension of some of the mechanisms regulating these muscle fiber type transitions, as well as muscle growth and repair, open the way for the development of new therapeutic approaches in respect to muscle wasting due to trauma, aging or disease.

Résumé

Il est reconnu depuis longtemps que le tissu musculaire squelettique est un organe hétérogène en termes de fonction et d'architecture. La recherche dans les domaines de la physiologie et de la biochimie ont permis le développement de nombreuses classifications basées sur des critères métaboliques ou ultrastructuraux décrivant différents types de fibres musculaires. Ces méthodes de discrimination se sont avérées plus ou moins corrélables entre elles selon les cas. De plus, il a été récemment démontré que les transitions de type de fibre musculaire en réponse aux changements environnementaux englobent un nombre croissant de protéines, de manière coordonnée ou non. Ce phénomène génère des types de fibres «atypiques» possédant des caractéristiques attribuées précédemment à des fibres distinctes, créant un continuum dans les échantillons analysés. Les stimuli induisant les changements de type de fibre ont également été étudiés, permettant de prédire plus ou moins précisément l'étendue et le type de transition obtenus dans des groupes musculaires définis en utilisant des protocoles standardisés.
Parallèlement, la compréhension de certains des mécanismes impliqués dans la régulation des transitions de type de fibre ainsi que dans la croissance et la régénération musculaire ouvre la voie au développement de nouvelles approches thérapeutiques applicables à la dégénération musculaire consécutive à un trauma, à l'âge ou à une maladie.

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Introduction

There are numerous types of movements one can achieve through skeletal muscle activity during everyday life or sport practice. The rapid withdrawal response after touching a hot object compared to the maintenance of the upright posture is only one example out of a multitude. This differentiation in locomotor response is sustained by the heterogeneity of skeletal muscle itself. As early as 1873, Ranvier documented the existence of red and white muscles *(Fig. 1a)*. A few years later, differences in fiber size and opacity were described *(Fig. 1b)*. During the last century, physiological recordings on isolated muscle fibers allowed separating them into fast and slow types *(Fig. 1c)*. The following decades, better understanding of the ultrastructure of skeletal muscle at the molecular level gave rise to fiber types delineation methods based on histochemical and biochemical characteristics. Classification of muscle fibers is mostly technique dependant though, which means, for example, that the attribution of a fiber type using a histochemical technique does not necessarily allow to deduct its contraction speed. As a matter of fact, the traditional concept of red-slow and white-fast muscle is not correct, as red-fast muscle fibers exist (so called fast oxidative glycolytic «FOG») (for review: [1]).

Muscle heterogeneity is a dynamic concept, as illustrated by the processes of atrophy or hypertrophy depending on training or disuse. The events occurring during these adaptive changes imply up- and down-regulation of molecules involved in the metabolism as well as in the ultrastructure of muscle cells, leading ultimately to recognizable differences in term of muscle fiber types. Some molecular mechanisms allowing muscle development and adaptation have already been elucidated. Myostatin (growth and differentiation factor 8) for example has been identified as the point of mutation in the so called double muscling (DM) bovines which exhibit an abnormally high proportion of muscle in comparison to bone and fat tissues (for review: [2]).

Muscle Fiber Types

One of the hallmarks of skeletal muscle tissue is its heterogeneity in term of fiber types, allowing for different functional capacities.

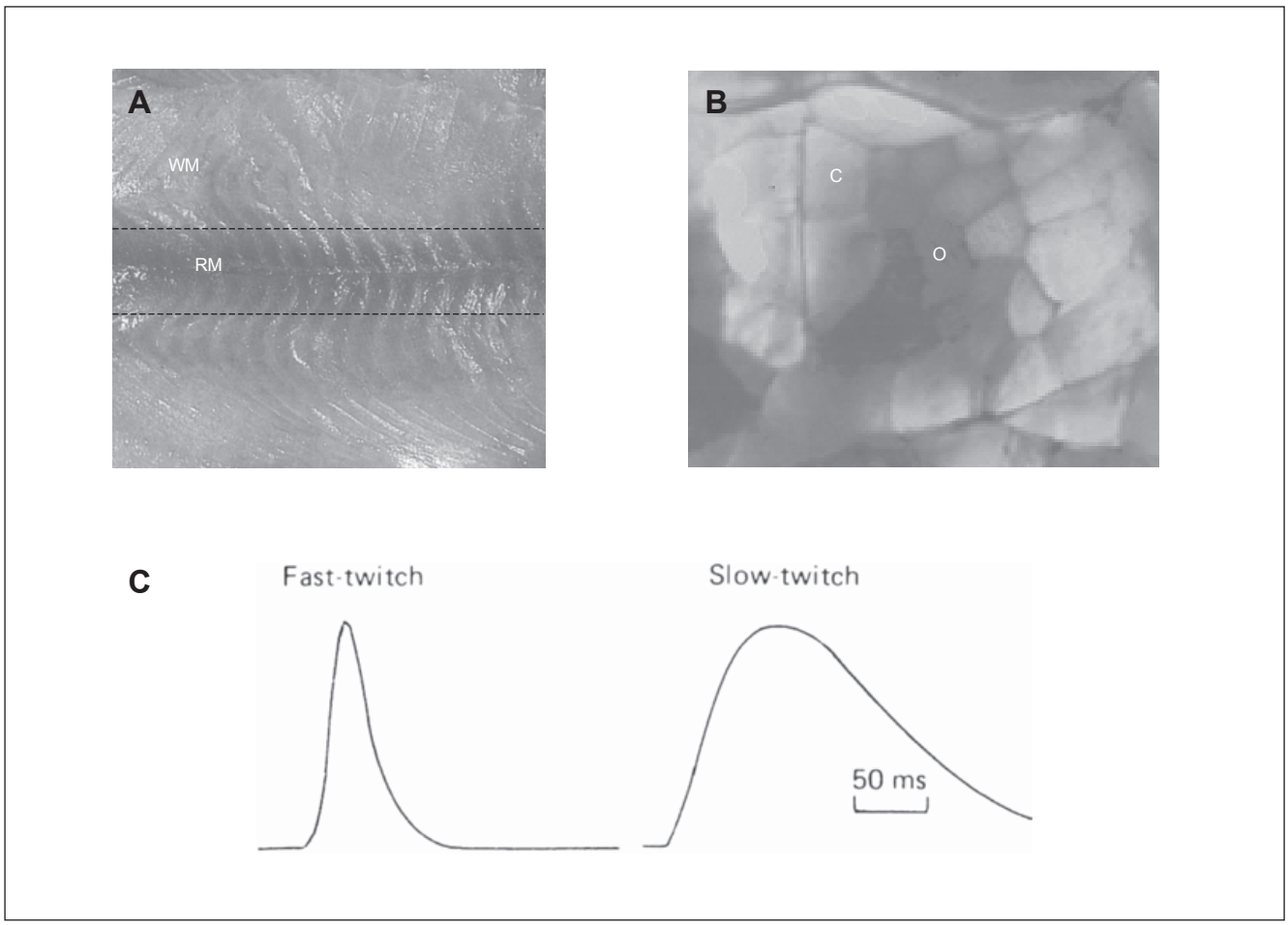

Figure 1: Muscle structural and biochemical properties.
A: Anatomical delineation between fish white (WM) and red (RM) muscle (Alexander R.McN.: Exploring Biomechanics. 1992, New York: Scientific American Library). *B:* Unstained skeletal muscle histological slide: clear (C) and opaque (O) zone reflecting myoglobin content of white and red muscle respectively. *C:* Examples of fast and slow twitch muscle contraction recordings (Keynes R.D., Aidley D.J.: Nerve and Muscle. 2001, Cambridge: Cambridge University Press).

This heterogeneity encompasses the wide range of skeletal muscle specific architecture and constitutive proteins *(Fig. 2)*. It has been studied using physiological, histological, biochemical or molecular biology techniques. Numerous classifications have evolved over the years leading to sometimes confusing nomenclatures.

Myosin heavy chain (MHC) based classification

The first widespread, qualitative, method developed was a histochemical classification of fibers based on myofibrillar actomyosin adenosine triphosphatase (mATPase) activity. A direct correlation with contraction velocity was shown, leading to the separation between slow, low mATPase activity: type I and fast, high activity: type II fibers [3]. Refinement in histochemical techniques, taking advantage of the acid and alkaline differential stability of the mATPase activity, led to a histochemical classification into seven muscle fiber types. The three main ones are one slow, type I (alkaline labile and acid stable) and two fast, type IIA and IIB (alkaline stable and acid labile) fibers. Intermediary types IC, IIC, IIAC and IIAB *(Fig. 3a)* also exist. Demonstration that mATPase is located on the globular part of the myosin heavy chain (MHC) [4] allowed to directly correlate MHC isoforms with muscle fibers shortening velocity [5]. Subsequently, development of molecular biology techniques allowed distinguishing between MHC isoforms using antibodies or electrophoretical separation *(Fig. 3b)*. This led to the identification of 11 MHC, of which 4 are widely distributed in adult muscle: MHC1β (slow) and MHCIIa, MHCIId and MHCIIb (fast). Fibers containing only one MHC complement are said to be «pure», the ones containing two or more are known as «hybrid». The four pure fibers corresponding to the principal MHC complements are the types Iβ, IIA, IID, and IIB. Intermediary types containing two isoforms were termed IC (MHC1β>MHCIIa), IIC (MHCIIa>MHC1β), IIAD (MHCIIA>MHCIID), IIDA (MHCIID>MHCIIA), IIDB (MHCIID>MHCIIB) and IIBD (MHCIIB>MHCIID) [6]. It is worth noting that numerous other myofibrillar proteins are quantitatively or qualitatively differentially expressed between fiber types. Myosin light chains, troponins, and sarco-endoplasmic reticulum calcium ATPase (SERCA) are only a few examples [7].

Metabolic enzyme based classification

Another common way of classifying muscle fibers is to quantify metabolic enzymes activity. Assessment of aerobic-oxydative (succinate dehydrogenase) and anaerobic-glycolytic enzymes activity (glycerophosphate oxidase) in combination with histochemical studies allowed functional separation of fibers into three types: slow oxidative (SO), fast oxidative and glycolytic (FOG) and fast glycolytic (FG) [1].

Correlations between techniques

The numerous existing approaches to delineate muscle fibers raised the question of compatibility between techniques. Studies trying to correlate mATPase characteristics with metabolic properties showed that although type I are the most oxidative and IIB the least, there is a wide overlapping range of activity within each specific fiber type [8].

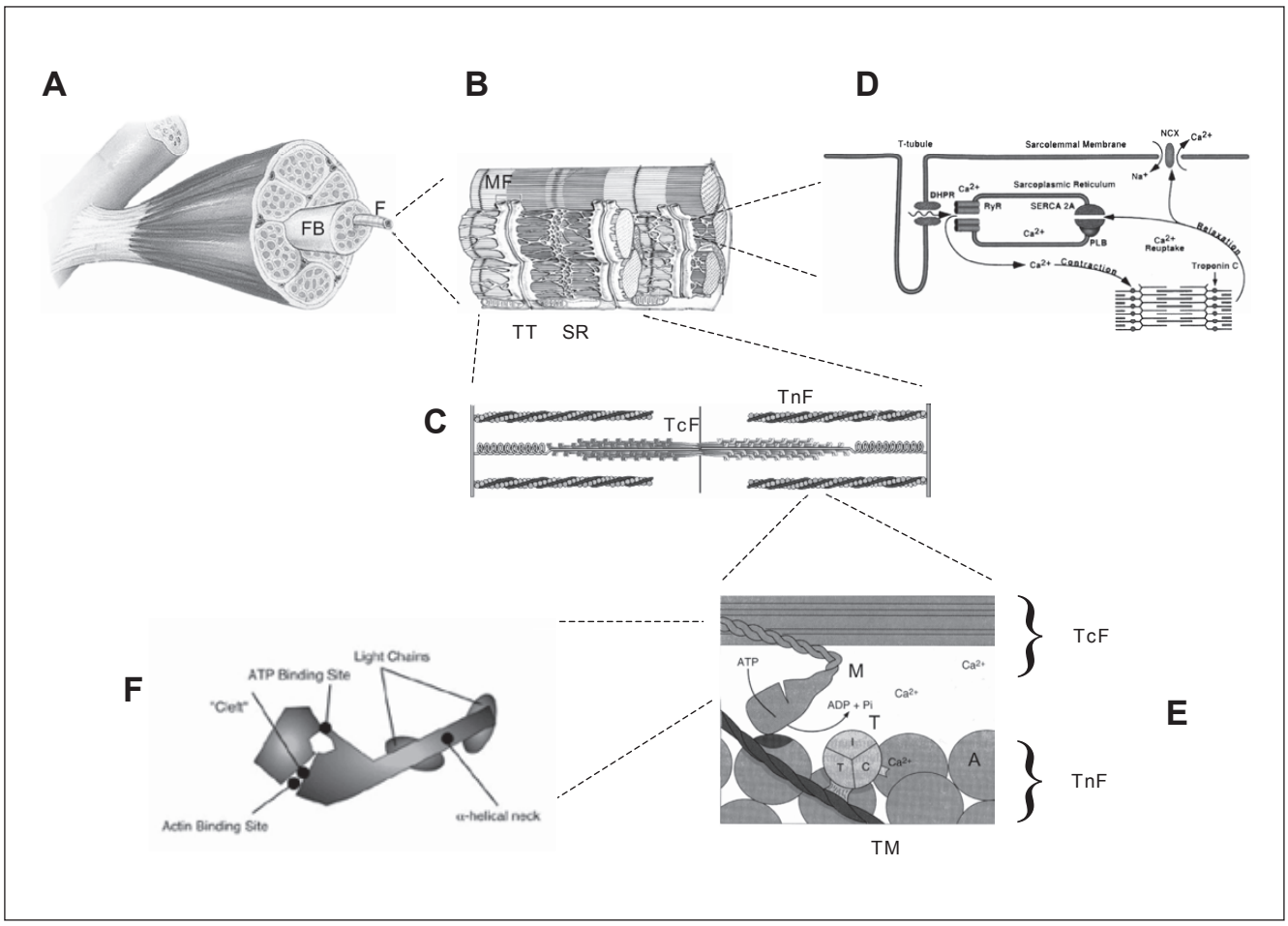

Figure 2: Histological and physiological classifications.
Individual fibers (F) in skeletal muscle arrange in fiber bundles (FB) *A*. They are composed of several myofibrils (MF), surrounded by the T-tubule (TT) system and sarcoplasmic reticulum (SR) *B* and containing the contractile protein of the thick (TcF) and thin filaments (TnF) *C*. Muscle contraction involves coupling between Dihydropyridine receptors (DHPR) and ryanodine receptors (RyR) leading to calcium release *D*. Ca^{2+} displaces tropomyosin (TM) from the myosin (M) binding site on actin (A) through a conformational switch of the troponin complex (T) *E*. This allows contraction at the expense of ATP hydrolysis (Ganong W.F.: Review of Medical Physiology. 1999, Stamford: Appleton & Lange). The myosin heavy chain head is composed of the globular part containing the ATPase and neck region binding the two light chains *F*.

On the other hand, MHC isoforms can be correlated to contractile properties when using stretch activation measurements on isolated myofibers. This technique permits separation of MHC complement based type I, IIA, IID and IIB pure fibers without overlap. Moreover hybrid fiber types are spread between the pure ones according to their specific isoform ratio [6]. Interestingly, the distribution of contractile velocities among fibers is paralleled by a corresponding distribution of mATPase activity and to isometric tension-cost ratios. Tension-cost recordings indeed increases gradually from type I (most efficient energetically speaking) through type IIB fibers [9].

Fiber Type Transitions

Model

Skeletal muscle is known to adapt to environmental factors. These include development, exercise, that can be separated in neuromuscular activity and mechanical constraints parts, hormonal profile and aging. Alterations take place in term of architecture as well as metabolic characteristics. They are achieved by qualitative and quantitative differential expression of several genes. These changes occur as coordinated gene expression programs in respect to MHC isoform switch for example *(Fig. 4)*. Studies are complicated by the fact that fiber type transitions generate atypical muscle fiber types. Metabolic enzymes activity can indeed be regulated by physical activity before changes can be observed at the MHC complement level. Furthermore, myosin light chain (MLC) isoforms switch does not necessarily follow the same kinetic as its MHC counterpart. This is also true for the other fiber type specific sarcomeric, cytoplasmic and membrane proteins [10]. Muscle fiber type transition appears therefore as a continuum encompassing all parts of the skeletal muscle cell apparatus in a more or less coordinated form.

Neuromuscular activity, exercise and mechanical loading

Alterations in muscle fiber composition can be obtained by modifying the neuromuscular input to muscle. Denervated fast muscles tend to become slower and denervated slow muscles tend to become faster. Cross re-innervation and electrostimulation experiments demonstrated the importance of the stimulus pattern to the fiber phenotype. Using chronic low frequency stimulation (CLFS) on fast muscles, simulating slow muscle activity, results in transitions through the full range of type IIB to type I fibers. Furthermore, it affects all functional elements of the muscle fiber (for review: [10]). Fast muscle innervation can be simulated using high frequency phasic stimulation. Slow to fast transitions only occur in the case of denervated muscles (for review: [11]).

Exercise training related modifications qualitatively parallel the ones obtained using CLFS. Changes, though, are of lesser amplitude and tend to be limited to the fast fiber types population,

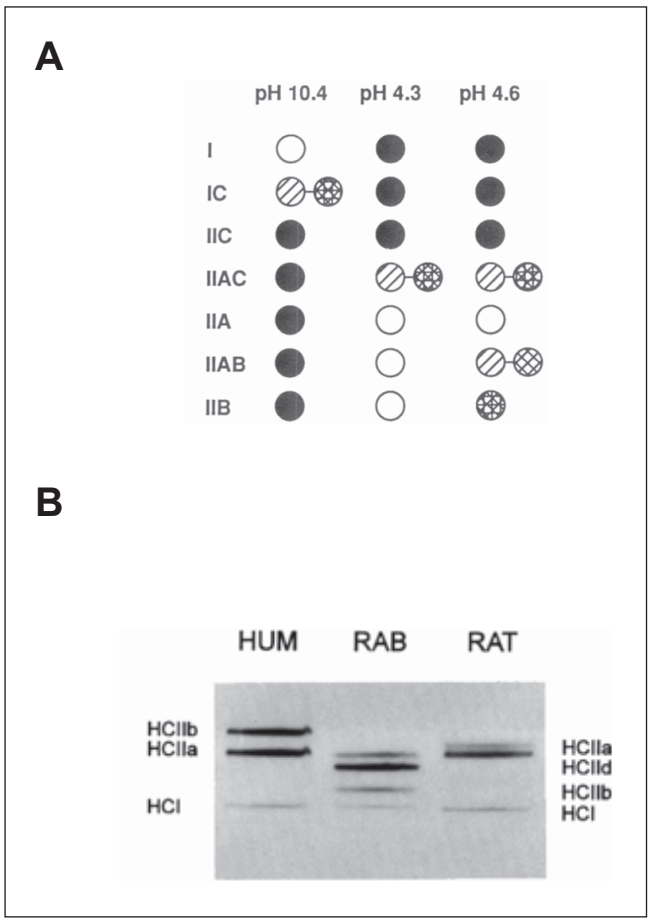

Figure 3: Histochemical classifications.
A: mATPase histochemical assay allows to delineate seven fiber types based on the pH stability of the enzyme. Slow type I ATPase are acid stable and alkaline labile, whereas fast fibers exhibit the opposite pattern (Staron R.S. and Hikida R.S.: J. Histochem. Cytochem. 40: 563–568, 1992). *B:* Electrophoretical separation (western blot) of myosin heavy chains in extracts from human pectoralis, rabbit gastrocnemius and rat diaphragm showing slow (HCI) and fast (HCIIa, HCIIb, HCIId) isoforms (Hamalainen N. and Pette D.: Patterns. Microsc. Res. Tech. 30: 381–389, 1995).

although reports of dose dependant, exercise induced, fiber II to I conversion exist [12]. Main changes, though, as evidenced in most studies, involve up-regulation of aerobic oxidative enzyme activities [13]. In parallel, it has been shown that exercise induced muscular changes include hyperplasia as well as hypertrophy [14].

Similar to CFLS, stretching or putting a muscle under mechanical constraint provokes fast to slow transition. The opposite is also true, as microgravity and hind-limb suspension, among other protocols, lead to slow to fast fiber switch [11].

Hormones and growth factors

Hormones and growth factors influence on muscle phenotype have been studied in research laboratories as well as used in the field by athletes, as evidenced by doping trials.

Testosterone for example has been shown to modify the fiber composition of defined muscle subsets [15] as well as their specific sex dependant fiber size [16] and even to determine the presence or absence of whole muscles as exemplified by the sexual dimorphism of the levator ani in rats [17].

Greatest effect though is achieved using thyroid hormones. Hypothyroidism induces fast to slow transitions and the opposite is true with hyperthyroidism (for review: [11]).

Recently, it has been shown that Insulin Growth Factor I (IGF-1) not only promotes muscle hypertrophy by stimulating muscle progenitor cells proliferation and differentiation, but it also helps

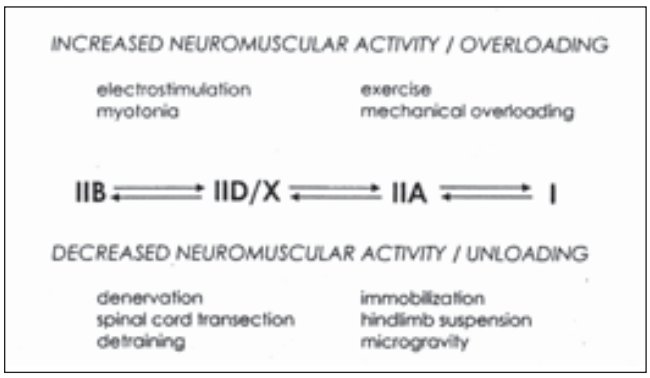

Figure 4: Muscle fiber type transitions.
Muscle fiber types transitions, as defined by their myosin heavy chain complement, occur through an ordered pathway from fast IIb to slow type I (Pette D. and Staron R.S.: Int. Rev. Cytol. 170: 143–223, 1997).

keeping the benefits of training in term of muscle hypertrophy during detraining phases [18].

Age

Aging is characterized by a reduction in skeletal muscle mass. This atrophy is paradoxically accompanied by a fast to slow transition [19]. Differentiating between primary and secondary changes in this context is difficult. Accompanying modifications include fiber type grouping, which suggests denervation-re-innervation cycles [6], as well as reduction in number and increase in size of motor units.

Mechanisms

Transition from one fiber type to another involves transcriptional, translational and post translational (proteolysis) modifications. The integration of these different alterations supposes the existence of control mechanisms. Possible candidates include the phosphorylation potential (ATP to ADP and AMP ratio) of muscle cells [20] as well as intracellular (activity dependant) Ca^{2+} levels [21] involving AMP activated protein kinase and Calcineurin respectively.

Intermediary between the activation of these pathways and changes of intracellular protein content are transcription factors. One of them, peroxisome-proliferator-activated receptor δ (PPAR δ) has been shown to induce macroscopically visible transitions (white to red muscle) from type II to type I muscle fibers when artificially expressed in mice. These changes where accompanied by strikingly increased physical performances as well as resistance to obesity [22].

Perspectives

Research on skeletal muscle in the past years uncovered the complexity of this organ and the refined mechanisms involved in its environmental adaptation. These discoveries encompass a wide range, going from the molecular structure of muscle itself to the numerous stimuli capable of altering its phenotype. Correlations between specific protein isotypes have been demonstrated and physiological and pharmacological stimuli inducing muscle hypertrophy and specific muscle fiber transitions have been isolated. This opens the way for new therapeutic techniques aimed at preventing muscle wasting consecutive to muscle trauma, age and degenerative diseases like Duchenne's muscular dystrophy. One can indeed foresee the use of gene therapy techniques applied to humans in order to have muscle targeted expression of proteins like IGF-1 or PPAR δ. These would lead to hypertrophy, increased benefit of exercise training and resistance to detraining in the former case and switch to type I fibers in the latter. At present, limiting

factors in the field of gene therapy are the availability of safe and efficient delivery vectors. Candidates include modified viruses like lentiviruses, able to efficiently transduce dividing as well as quiescent cells, as is normally the case with muscle fibers. Other means of delivery include myogenic precursor cells themselves, which have been shown to be capable of being efficiently transduced with coding sequences for specific proteins and express their transcript after integration in muscle fibers. This has been demonstrated in small as well as in big, closer to human, animal models [23]. It is also easy to predict that such therapies might not only address the population of sport medicine patients but also the one of healthy athletes subjected to competitive pressure. In this way, use of gene therapy as a doping technique might complicate the fight against cheating in sports, as it is not readily detectable using standard tests (for review: [24]). Furthermore, increasing evidence of inter-individual inherited differences in term of muscle performances will raise the question of reevaluating competition categories according to genetic background. The first case of a boy born with a natural mutation concerning the myostatin gene has indeed been published [25], and there is little doubt that if this boy chooses to compete in power lifting, his future opponents will have to train hard to keep the pace.

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