Training effects on the contractile apparatus

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ABSTRACT

Skeletal muscle is an extremely heterogeneous tissue, composed of a large variety of fibre types. Its dynamical nature is reflected by the ability to adapt to altered functional demands by qualitative alterations in fibre type composition. The molecular basis of this versatility is that specific myofibrillar and Ca²⁺-regulatory protein isoforms are assembled to functionally specialized fibre types. Based on this diversity, adult muscle fibres are capable of changing their molecular composition by altered gene expression. Myosin heavy chain (MHC) isoforms and their unique expression in 'pure' fibres, as well as their coexpression in 'hybrid' 'fibres' represent the best markers of muscle fibre diversity and adaptive changes. Chronic low-frequency stimulation (CLFS) and endurance training represent highly suitable models for studying the effects of increased neuromuscular activity on myofibrillar protein isoform expression and fibre type composition. Generally, both models induce fast-to-slow transitions in myofibrillar protein isoforms and fibre types. However, the responses to endurance training are quantitatively less pronounced than those in muscles exposed to CLFS. Parallel changes in isoforms of specific myofibrillar or Ca2+-regulatory proteins during the induced fast-to-slow transitions point to the existence of fibre type-specific patterns of gene expression. The fast-to-slow transitions do not proceed in abrupt jumps from one extreme to the other, but occur in a gradual and orderly sequential manner. Depending on the basal protein isoform profile, and hence the position within the fast-slow spectrum, the adaptive ranges of different fibre types vary. However, adaptive ranges not only depend on a particular fibre type, but also are influenced by species-specific properties.

Keywords Ca²⁺-regulatory proteins, electrical stimulation, exercise training, fibre type transition, myofibrillar proteins, myosin, protein isoforms, skeletal muscle.

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Skeletal muscle is an extremely heterogeneous tissue. Its heterogeneity is reflected by its composition of a large variety of diverse fibre types. Moreover, skeletal muscle is characterized by a highly adaptive potential. This property is due to the functional plasticity of muscle fibres, which are versatile elements and capable of modifying their molecular composition and properties. Both, the diversity and versatility of muscle fibres relate to the particularity that most proteins of the contractile apparatus, Ca²⁺-handling, and energy metabolism exist as sets of multiple isoforms. This multiplicity at the level of sarcomeric protein isoforms, as well as the modular design of sarcomeres and myofibrils provide the basis for the modelling and remodelling of a large spectrum of fibre types to match specific requirements and to adapt to altered functional demands. In order to discuss training effects on the contractile apparatus, it will be necessary to first review patterns of myofibrillar protein isoforms in specific muscle fibre types. This will be the basis for discussing experimentally induced changes in myofibrillar protein isoform patterns and fibre types, especially with regard to the effects of chronic low-frequency stimulation (CLFS) and endurance exercise training.

MYOFIBRILLAR PROTEIN ISOFORMS

Myosin light and heavy chains, isomyosins, and myosin-based fibre types

Multigene families and alternative transcript splicing create multiple thick and thin filament protein isoforms (Table 1). These may be assembled in specific patterns to generate functionally specialized fibre types. Myosin, the major protein of the thick filament, is currently considered as the most suitable marker of fibre type diversity. Myosin heavy chains (MHC), as well as my-

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 Table 1
 Major myofibrillar protein isoforms in extrafusal fibres of limb muscles in small mammals

	Slow	Fast
Myosin heavy chains	ΜΗCΙβ, ΜΗCΙα, ΜΗCΙa	MHCIIa, MHCIId(x), MHCIIb
Myosin light chains		
Alkali	LC1sa, LC1sb	LC1f, LC3f
Regulatory	LC2s	LC2f
Tropomyosin	TMas < TM β	$TM\alpha f > TM\beta$
Troponin		
Troponin-T	TnT1s, TnT2s	TnT1f, TnT2f,
		TnT3f, TnT4f
Troponin-I	TnIs	TnIf
Troponin-C	TnCs	TnCf

osin light chains (LC) exist as fast and slow isoforms. To date, at least 10 MHC isoforms have been identified in adult extrafusal fibres (Pette & Staron 1990, Schiaffino & Reggiani 1996). The major fast isoforms present in limb muscles of small mammals are MHCIIb, MHCIId(x), and MHCIIa. They are found in the fast fibre types IIB, IID(X), and IIA, respectively. Additional fast MHC isoforms exist, but their distribution is restricted to specific muscles, e.g. extraocular and masticatory muscles. The abundant MHC isoform in slow-twitch muscles is the slow MHCI β thought to be identical to the β -MHC of cardiac ventricle (Lompré et al. 1984). Fibres containing MHCI are designated as type I. Recent observations on rabbits indicate that an α -cardiac-like MHC isoform (MHCI α) is expressed also in slow fibres of limb muscles and the diaphragm (Peuker & Pette 1995, Hämäläinen & Pette 1997b). Combined biomechanical and biochemical studies have recently identified an additional slow MHC isoform in rabbit plantaris muscle which contains a fraction of type I fibres characterized by the coexistence of MHCI β and an additional slow isoform, MHCIa (Galler et al. 1997a). As a result of its slightly faster mobility, the latter can be separated electrophoretically from MHCI β (Fauteck & Kandarian 1995, Hämäläinen & Pette 1996). Further studies are necessary to determine the distribution of MHCIa and MHCIa in other muscles and in animal species. The number of MHC isoforms is growing and with the aid of more refined analytical methods and by investigating a greater variety of muscles, it will continue to grow. According to studies on chicken, the MHC multigene family encompasses 31 members (Robbins et al. 1986).

Comparative studies on small mammals point to species-specific differences suggesting that increasing body size shifts the profile of the fast MHC isoforms from MHCIIb towards MHCIId(x) and MHCIIa (Hämäläinen & Pette 1995). In other words, fast-twitch muscles of larger animals contain lesser type IIB fibres than do homologous muscle of smaller animals. This observation is in agreement with the finding that the fastest MHC isoform in human muscle is not MHCIIb but is homologous to the MHCIId(x) (Sant'Ana Pereira & Moorman 1994, Smerdu *et al.* 1994, Ennion *et al.* 1995).

Based on myosin immunohistochemistry and single fibre analysis of MHC isoforms, at least four pure fibre types, uniquely expressing a single MHC isoform, can be delineated in limb muscles of small mammals. According to measurements of unloaded shortening velocity, myofibrillar ATPase activity, and stretch-activation in single rat muscle fibres, the speed characteristics of these fibre types decrease in the order of type IIB > type IID(X) > type IIA \geq type I (Bottinelli *et al.* 1991, 1994a, b, Galler *et al.* 1994, 1996, Hilber *et al.* 1997). A similar order of fibre types has been demonstrated for human muscle (Larsson & Moss 1993).

Hybrid fibres coexpress two or more MHC isoforms (Pette & Staron 1990). Thus, a continuum exists which spans between the very fast type IIB and the slow type I fibres and in which pure fibres are bridged by hybrid fibres expressing their next-neighbour MHC isoforms (Staron & Pette 1993). Pure and hybrid fibre types may also be delineated by their histochemically assessed myofibrillar ATPase (mATPase) activity (Hämäläinen & Pette 1993).

Although the light chains do not seem to appreciably affect the actin-activated myosin ATPase activity, they have a significant impact on the shortening velocity (Lowey et al. 1993, Bottinelli et al. 1994b). This influence is of interest in view of the existence of various isomyosins. The existence of two fast alkali light chains (LC1, LC3) generates three combinatorial patterns, an LC1 homodimer, an LC1/LC3 heterodimer, and an LC3 homodimer. Their combination with a pair of regulatory light chains and an MHC homodimer results in three electrophoretically distinct isomyosins, FM1, FM2, and FM3 (d'Albis & Gratzer 1973, Hoh & Yeoh 1979). Taking into account the existence of three fast MHC isoforms, a total of nine fast isomyosins is present in limb muscle fibres, three MHCIIb-based (FM1b, FM2b, FM3b), three MHCIId(x)-based (FM1d, FM2d, FM3d), and three MHCIIa-based (FM1a, FM2a, FM3a) (Termin & Pette 1991, Wada et al. 1995). As a result of the existence also of two slow alkali light chains isoforms (LC1sa, LC1sb), three MHCI β -based slow isomyosins have been distinguished electrophoretically (Termin & Pette 1991). In addition, MHCIabased isomyosins have been detected in rabbit muscles (Hämäläinen & Pette 1997b).

The functional significance of variations in the relative amounts of the two alkali light chains has been studied in fast fibre subtypes (Greaser *et al.* 1988, Bottinelli *et al.* 1994a, Moss *et al.* 1994, Bottinelli & Reggiani 1995). The LC3/LC1 ratio was found to finely tune the shortening velocity, while coarse adjustment of shortening velocity is achieved by different MHC isoforms. Thus, an even greater multiplicity is created at the isomyosin level. Obviously, the number of isomyosins increases in hybrid fibres, especially by coexistence of fast and slow light chain isoforms in combination with one or more MHC isoforms.

Regulatory proteins of the thin filament and calcium-handling proteins

Actin, the major protein of the thin filament, is uniquely present as the α -skeletal isoform in adult muscle fibres, whereas the regulatory proteins, tropomyosin (TM) and troponin (Tn), exist as fast and slow isoforms (Pette & Staron 1990) (Table 1). TM is a dimer composed of muscle-specific α -TM and β -TM subunits and exists as α/α and β/β homodimers or as α/β heterodimer. The β -subunit is predominant in slow fibres which also contain a slow isoform of the α -subunit.

The inhibitory (TnI) and Ca²⁺-binding (TnC) subunits of troponin exist as slow and fast isoforms in slow and fast fibres, respectively. The molecular diversity of the TM-binding troponin subunit (TnT) extends beyond merely single fast and slow isoforms. At least four major fast (TnT_{1f}, TnT_{2f}, TnT_{3f}, TnT_{4f}) and two slow (TnT_{1s}, TnT_{2s}) isoforms have been identified (Moore *et al.* 1987, Briggs & Schachat 1989, Härtner *et al.* 1989). Studies on rat muscle revealed fibre type-specific expression patterns with the following preferential combinations of TnT and MHC isoforms: (MHCIIb + TnT_{4f} > TnT_{2f}), (MHCIId + TnT_{1f} > TnT_{2f}), (MHCIIa + TnT_{3f} > TnT_{2f}), (MHCI + TnT_{1s} > TnT_{2s}) (Galler *et al.* 1997c).

Calcium-regulatory proteins

Only some of the Ca²⁺-handling proteins exist as slow or fast isoforms. No fibre type-related isoforms of the Ca²⁺-release channel (dihydropyridine and ryanodine receptors) have to date been detected (Meissner & Lu 1995). Conversely, the Ca²⁺-pumping ATPase of the sarcoplasmic reticulum (SR) is present in adult fast and slow fibres as fast (SERCA1a) and slow (SERCA2a) isoforms (for a review see Dux 1993). Finally, two proteins functionally related to Ca²⁺-sequestration display fibre type-specific distribution patterns, without existing as isoforms. Phospholamban, a regulatory protein of the SR Ca²⁺-ATPase is present only in cardiac muscle and slow-twitch fibres (Jorgensen & Jones 1986). Parvalbumin, a Ca²⁺-binding protein functionally interposed between troponin-C and the SR Ca²⁺-ATPase is expressed at high amounts in fast-twitch, but not in slow-twitch muscles of lower vertebrates and small mammals (Heizmann et al. 1982). Its concentration is

highest in type IIB, lowest in type IIA, and intermediate in type IID(X) fibres (Schmitt & Pette 1991).

FIBRE TYPE TRANSITIONS

Muscle fibres are dynamical structures and capable of rearranging their molecular composition in response to exogenous signals. Depending on the quality of the signal, its intensity and duration, some or all functional elements of the muscle fibre may be affected. Adaptive changes must not necessarily occur in synchrony because differences in thresholds may exist at which different functional elements of the muscle fibre undergo alterations. For example, enzyme activities of aerobicoxidative metabolic pathways may increase in exercising muscle without noticeable MHC-based fibre type transitions (Jacobs-El et al. 1993, Pette & Staron 1997). Obviously, the contractile machinery is not tightly coupled to a specific type of energy metabolism. Conversely, parallel changes in the transitions of MHC and TnT isoforms point to a tight coupling of these two elements (see below).

The phenotypic properties of muscle fibres are controlled by various factors. Some are important during development, e.g., cell lineage-specific programmes and innervation (Gunning & Hardeman 1991). Others, such as neuromuscular activity and usage patterns have strong effects on adult fibres. Generally, increased neuromuscular activity tends to induce fastto-slow transitions, whereas decreased neuromuscular activity causes transitions in the opposite direction (for a review see Pette & Staron 1997).

Chronic low-frequency stimulation and endurance exercise training have been most frequently used for studying the effects of enhanced neuromuscular activity. Both protocols lead to transitions in the fast-toslow direction, but CLFS has the stronger effect.

Fast-to-slow transitions by CLFS

Compared to exercise training, CLFS offers the following advantages: (1) a reproducible and standardized regimen of contractile activity; (2) uniform activation of all motor units in the stimulated muscle; (3) stimulation-induced responses disclose the full range of possible adaptive changes; (4) no acclimatization of the animal is necessary; (5) maximum contractile activity is applied with the onset of stimulation and, therefore, changes in molecular, structural, and functional properties can be followed from the very beginning.

Indirect stimulation of a fast-twitch muscle with an impulse pattern similar to that normally delivered to a slow muscle results in an orderly sequence of changes affecting all functional elements of the muscle fibre: the contractile and regulatory proteins of the thick and thin filaments, the proteins of the Ca²⁺-regulatory system, as well as enzyme activity and isozyme patterns of energy metabolism (for a review see Pette & Vrbová 1992). The common protocol for CLFS of fast-twitch extensor digitorum longus (EDL) and tibialis anterior (TA) muscles in small mammals (rat and rabbit) uses a 10-Hz impulse pattern (single pulse duration 20 ms) delivered to the peroneal nerve via laterally fixed electrodes. The wires are led subcutaneously to the back of the animal and connected to a stimulator or, as in the case of telestimulation (Schwarz *et al.* 1983), to a little receiver fixed to the animals's back. Depending on the specific aim of the experiment, stimulation may be continuous (24 h day⁻¹) or discontinuous (12 h day⁻¹, i.e. alternating 1-h periods of stimulation and rest).

Fast-to-slow transitions of myosin isoforms

At the level of the thick filament, there is a gradual fastto-slow transition by sequential exchanges of MHC isoforms. In the fast-twitch TA and EDL muscles of rabbits, this process follows the order of MHCIId(x) \rightarrow MHCIIa \rightarrow MHCI β (Fig. 1) (Staron *et al.* 1987, Leeuw & Pette 1993). Recent studies indicate the α -cardiac-like MHCI α to be transiently expressed as an intermediate step in the final fast-to-slow conversion (Peuker & Pette 1995, Hämäläinen & Pette 1997b, Peuker *et al.* 1998).

Depending on the muscle stimulated, the animal species used, and the duration of CLFS, sequential transitions in MHC expression may start and end at different stages of the fast-to-slow transformation. Furthermore, the time points for the onset of MHC isoform transitions may vary for different animal species. Thus, rat TA and EDL muscles with a high proportion of type IIB fibres begin their stimulation-induced exchange of MHC isoforms with a MHCIIb to MHCIId(x) transition. This exchange seems to coincide with a MHCIId(x) to MHCIIa transition. As illustrated in Figure 2, MHCIIb decreases abruptly and MHCIIa rises to become the predominant MHC isoform in muscles stimulated for longer than 40 days. Initially, the relative concentration of MHCIId(x) remains unaltered, but starts to decline by 30 days. Contrary to TA muscle of rabbit, where MHCI β rises to approximately 65% of the total MHC isoforms after 60 days (Leeuw & Pette 1993), the relative concentration of MHCI is only moderately (~10-15% relative concentration) elevated in rat TA muscle exposed to long-term (100 days) stimulation (Fig. 2). As shown by mATPase histochemistry, the percentage of type I fibres amounts to less than 10% in 60-day stimulated EDL muscle of rats (Delp & Pette 1994). It is evident, therefore, that the degree of the fastto-slow transformation is much higher and occurs much earlier in rabbits than in rats (Leeuw & Pette 1993,

Hämäläinen & Pette 1997a). Finally, the CLFS protocol, which elicits increases of type IIA fibres in rats and of type I fibres in rabbits, has only small effects in mouse. Mouse TA and EDL muscles exposed to CLFS for up to 35 days exhibit only some type IIB to type IID(X) transitions, but no increase in type IIA fibres (A. Termin, N. Hämäläinen & D. Pette, unpublished observations). It is much easier, therefore, to transform fast muscles into slow muscles in rabbits than in rats and mice. We have previously shown that the fast-to-slow transitions in rat muscle can be enhanced by lowering the thyroid hormone level. Hypothyroidism favours the expression of MHCIIa and MHCI in low-frequency stimulated rat muscle, whereas elevated levels of the hormone have an antagonizing effect (Kirschbaum *et al.* 1990).



Figure 1 Sequential exchange of MHC isoforms in low-frequency (10 Hz) stimulated tibialis anterior muscle of the rabbit. Relative MHC concentrations were determined by densitometric evaluation of silver-stained gradient gels from extracts of muscles stimulated for different periods of time. Values are means \pm SD, n = 3-5 for each time point. (∇), MHCIId(x); (\blacktriangle), MHCIIa; (\odot), MHCI. Adapted from Leeuw & Pette (1993).



Figure 2 Sequential exchange of MHC isoforms in low-frequency (10 Hz) stimulated tibialis anterior muscle of rats. Relative MHC concentrations were determined by densitometric evaluation of silverstained gradient gels from extracts of muscles stimulated for different periods of time. Data are from one animal per time point. (\Box), MHCIIb; (∇), MHCIId(x); (\blacktriangle), MHCIIa; (\odot), MHCI. (B. Gohlsch & D. Pette, unpublished observations).

Stimulation-induced changes in MHC isoform composition have also been studied at the mRNA level (Jaschinshi *et al.* 1998). The results clearly demonstrate that the changes in the amounts of the different MHC mRNA isoforms precede the corresponding changes at the protein level. mRNAs specific to slow MHC isoforms were shown in rabbit fast-twitch muscle to rise only after stimulation periods of 20 days (Peuker & Pette 1995), whereas significant increases in MHCI protein were detected in 35-day stimulated muscles (Leeuw & Pette 1993). In rat fast-twitch muscles, however, increases in MHCI β mRNA were not detected during CLFS for up to 42 days (Jaschinshi *et al.* 1998). These results further add to the notion of species-specific ranges of adaptation (Simoneau & Pette 1988).

A species-specific pattern of adaptation seems to emerge also from studies in humans. MHC analyses on single fibres from the vastus lateralis muscle of spinalcord injured patients exposed to electrical stimulation for of up to 1 year, displayed pronounced increases in MHCIIa, but never in MHCI (Andersen et al. 1996). However, these results may not be comparable to the animal studies because of the use of different stimulation protocols and impulse patterns. The failure to induce fast-to-slow transitions beyond the enhanced expression of MHCIIa may also result from the lack of tensile load by antagonists in the paralysed patients. The importance of stretch in accelerating stimulation-induced fast-to-slow transitions has been demonstrated in several animal studies, e.g. (Goldspink et al. 1992, 1995, Pattullo et al. 1992).

The exchange of MHC isoforms in transforming muscle leads to an expanded population of hybrid fibres. For example, approximately 60% of the fibre population in 30-day stimulated rabbit TA muscle are C fibres containing both MHCIIa and MHCI (Staron et al. 1987). Depending on the state of transformation, the combinations of MHC isoforms coexisting in single fibres differ and change with ongoing stimulation, but generally encompass nearest neighbour isoforms, i.e. MHCIIb + MHCIId(x), MHCIId(x) + MHCIIa, and MHCIIa + MHCI (Staron et al. 1987, Termin et al. 1989, Aigner et al. 1993). Coexistence of more than two MHC isoforms is possible, especially when different MHC isoform exchanges coincide, e.g. in rat muscle during the initial phase of transformation (Fig. 2). Coexistence of several MHC isoforms may also arise from nonuniform MHC isoform expression along transforming fibres (Staron & Pette 1987).

The replacement of fast myosin light chains with their slow counterparts is in agreement with the overall fast-toslow transition at the MHC level. However, these changes do not occur in a synchronous manner. The exchange of the fast regulatory light chain LC2f with the slow LC2s precedes the fast-to-slow transitions of the alkali light

chains (Brown et al. 1983, Seedorf et al. 1983, Leeuw & Pette 1996). Taking into consideration the MHC isoform transitions, asynchronous changes in the light chain complement must give rise to a vast number of isomyosins. Thus, in 60-day stimulated TA muscle of rabbits the slow MHCI content (~65%) exceeds by far that of the slow alkali LC1s (~25%) (Fig. 3a, b) (Leeuw & Pette 1996). Therefore, an appreciable number of MHCI molecules must associate with fast alkali light chains. Hybrid myosins may also originate from other combinations of fast and slow isoforms. Thus, MHCIIa predominates (>90%) in 35-day stimulated rabbit TA muscle in which the relative concentration of MHCI amounted to only 5% (Leeuw & Pette 1996) (Fig. 3b). In the same muscles, the slow light chains LC1s and LC2s range between 10% and 15%, respectively. Therefore, some MHCIIa molecules must combine with slow light chains. Obviously, MHCI and MHCIIa are capable of associating with fast and slow light chain isoforms.

Fast-to-slow transitions of regulatory proteins of the thin filament

Chronic low-frequency stimulation-induced fibre type transitions also modify the isoform pattern of thin filament proteins (Härtner *et al.* 1989, Leeuw & Pette 1993). Time course studies on rabbit TA muscles stimulated for up to 60 days have indicated a close relationship between the transitions of troponin T and MHC isoforms (Leeuw & Pette 1993). The initial decrease in MHCIId(x) occurred in parallel with decreases in TnT_{1f} and TnT_{2f} , while the increase in MHCIIa was concomitant with that of TnT_{3f} . The ultimate increase in slow MHCI was in parallel with the rise of the two slow TnT isoforms, TnT_{1s} and TnT_{2s} .

Chronic low-frequency stimulation also induces a shift towards the slow isoforms of TnI and TnC. However, even after 60 days of CLFS the percentages of slow TnI and TnC do not exceed 40–45% (Fig. 3c). Interestingly, these slow troponin isoforms start to rise at 35 days when only low amounts of MHCI were expressed (Leeuw & Pette 1993). These observations indicate that some type IIA fibres of the transforming muscle must contain hybrid troponin molecules composed of TnT3f combined with fast or slow isoforms of TnI and TnC. Similarly, fibres, which contain MHCI and slow TnT in combination with fast TnI and TnC isoforms, must exist.

Fast-to-slow transitions of calcium-regulatory proteins

As may be expected, CLFS also leads to profound changes in the Ca^{2+} -regulatory system. A major change relates to the sarcoplasmic reticulum Ca^{2+} -ATPase with an isoform switch from the fast SERCA1a to the slow



Figure 3 Myosin light chain (a), myosin heavy chain (b), and troponin subunit (c) isoform composition of unstimulated (control), 35-day, and 60-day stimulated rabbit tibialis anterior muscle. In the case of the myosin light chains and the troponin subunits, the relative concentrations of slow isoforms are expressed as percentages of the total isoforms. The values (means \pm SD) are from Leeuw & Pette (1993).

SERCA2a (Leberer *et al.* 1989, Ohlendieck *et al.* 1991, Hu *et al.* 1995, Hicks *et al.* 1997). A high correlation was detected between relative concentrations of SERCA2a and slow MHCI isoforms in low-frequency stimulated rabbit EDL and TA muscles, suggesting a coordinated expression of slow myosin and slow Ca²⁺-ATPase isoforms (Hämäläinen & Pette 1997a). Closely related expression patterns can also be deduced from the steep decrease in protein amounts of the dihydropyridinesensitive Ca²⁺ channel of the T-tubules and the ryanodine-sensitive Ca²⁺-release channel of the junctional SR (Ohlendieck *et al.* 1991). Triadine declines in parallel, suggesting its regulation to be coordinated with these key components of EC-coupling (Hicks *et al.* 1997).

Another effect of CLFS on the Ca²⁺-regulatory system is the induction of phospholamban, normally not expressed in fast-twitch muscle (Leberer et al. 1989, Briggs et al. 1992, Hu et al. 1995). The induction of phospholamban occurs in a coordinate manner with SERCA2a (Hu et al. 1995). Conversely, parvalbumin, normally not expressed in slow-twitch muscle, is suppressed by CLFS (Leberer & Pette 1986, Leberer et al. 1986, Huber & Pette 1996). According to its fibre typespecific distribution, its decrease is only moderate during the type IIB \rightarrow type IID(X) transition, but is markedly enhanced during the subsequent type IID (X) \rightarrow type IIA transition. According to studies on rat TA muscle, the decrease in parvalbumin occurs much later than its mRNA (Huber & Pette 1996). The adjustment of parvalbumin levels during the sequential fibre type transitions, therefore, seems to involve post-translational regulatory mechanisms where protein degradation might be a limiting step.

Exercise training

Most studies on the effects of exercise training in skeletal muscle deal with metabolic effects of sustained contractile activity. Generally, the metabolic responses to endurance training are qualitatively similar but quantitatively less pronounced than those in electrically stimulated muscles (Pette & Vrbová 1992). The same holds true for studies on the effects of exercise training on myofibrillar proteins and fibre types. This seems to relate to the fact that any type of exercise training exposes skeletal muscles to much smaller amounts of contractile activity than CLFS. Moreover, when comparing effects of CLFS and exercise training species-specific ranges of adaptation should be taken into consideration. In addition, the extent of inducible changes depends on the initial molecular and cellular profile of a given muscle. Thus, a type IIB fibre ultimately transforming into a type I fibre has a wide adaptive range whereas a type IIA fibre has a limited range with regard to fast-to-slow transition.

In general, studies on training-induced changes in myofibrillar protein composition and fibre types encompass fewer parameters than studies using CLFS. Many studies on endurance training in humans have demonstrated by mATPase histochemistry increases in the fraction of type IIA fibres with concomitant decreases in type IIB fibres (Andersen & Henriksson 1977, Jansson & Kaijser 1977, Green *et al.* 1979, Ingjer 1979, Martin III *et al.* 1989, Harridge 1996). Likewise, endurance training in rats has been shown to cause a decrease in MHCIIb together with an increase in MHCIIa (Sugiura *et al.* 1992). In addition, several endurance training studies in humans and rats have reported fibre transformations beyond type IIA, i.e. increases in hybrid C fibres (Jansson & Kaijser 1977, Schantz & Henriksson 1983, Schantz & Dhoot 1987, Klitgaard et al. 1990a), type I fibres (Green et al. 1984, Luginbuhl & Dudley 1984, Howald et al. 1985, Simoneau et al. 1985, Goubel & Marini 1987), and slow isomyosin (Fitzsimons et al. 1990). Exercise-induced fast-to-slow transitions have also been observed in rats and humans at the myosin light chain level and with regard to reduced parvalbumin content (Green et al. 1984, Baumann et al. 1987).

Fibre type transitions in response to both sprint and resistance training appear to resemble qualitatively those observed in endurance training. Sprint training in rats and humans has been shown to cause an increase in type IIA fibres and a decrease in type IIB fibres with accompanying changes in MHC content (Klitgaard *et al.* 1990a, Morales-López *et al.* 1990, Jacobs *et al.* 1987, Allemeier *et al.* 1994, Andersen *et al.* 1994b). Similarly, strength training in humans has been shown to result in type IIB \rightarrow type IIA fibre transitions (Klitgaard *et al.* 1990b, Staron *et al.* 1990, 1994, Hather *et al.* 1991, Andersen *et al.* 1994a) and corresponding changes in MHC complement (Adams *et al.* 1993, Andersen *et al.* 1994a, Staron *et al.* 1994).

POSSIBLE MECHANISMS UNDERLYING FIBRE TYPE TRANSITIONS

Skeletal muscle responds to altered functional demands by specific qualitative and quantitative alterations in gene expression, provided that the stimuli are of sufficient magnitude and duration. Persistently elevated neuromuscular activity induces a series of concerted changes in gene expression, effecting fast-to-slow transitions in muscle fibre phenotypes. During these graded and orderly sequential transitions, some of the changes in myofibrillar protein isoforms occur in parallel, suggesting fibre type-specific programs of gene expression, e.g. fast and slow MHC and TnT isoforms, MHCI and SERCA2a, phospholamban and SERCA2a, or parvalbumin and MHCIIb/MHCIId(x) isoforms.

It is now clear that MHC isoforms represent the best possible marker of fibre type transitions. For mammalian limb muscles, the spectrum of MHC isoforms spans from MHCIIb on one end to MHCI β on the other end. The changes in MHC isoform expression seem to occur in a sequential order. Thus, a type IIB fibre ultimately transforming into a type I fibre does not switch directly from MHCIIb to MHCI β , but will express MHCIId(x) and MHCIIa before expressing MHCI β . In rabbits, these sequential transitions also involve MHCI α as an additional step between MHCIIa and MHCI β (Peuker & Pette 1995, Hämäläinen & Pette 1997b, Peuker *et al.* 1998).

Interestingly, this sequence of MHC isoform transitions originally deduced from studies on low-fre-

quency stimulated muscle (Staron et al. 1987, Termin et al. 1989, Leeuw & Pette 1993), corresponds to an order derived from differences in myofibrillar ATPase activity, unloaded shortening velocity, tension cost, and stretch-activation of pure and hybrid fibres in normal muscles (Bottinelli et al. 1991, 1994a, b, Larsson & Moss 1993, Galler et al. 1994, 1997b, c, Hilber et al. 1997). Of these, the tension cost, defined as the ratio between ATPase activity and isometric tension, seems to be highly relevant with regard to the functional order of MHC isoforms and fibre types. According to Bottinelli and coworkers, type IIB fibres display the highest tension cost, fibre types IID(X) and IIA are intermediate, and type I fibres the lowest (Bottinelli et al. 1994b). These data suggest that transitions from one MHC isoform to another might follow energetic requirements. Therefore, the energy potential of the muscle fibre may play an important regulatory role in determining the MHC isoform profile. Measurements of the [ATP]/[ADP_{free}] ratio in low-frequency-stimulated rabbit muscles and in single transforming fibres support the hypothesis that MHC isoform expression is influenced by the cellular energy potential (Green et al. 1992) (A. Conjard & D. Pette, unpublished observations). Low-frequency stimulation causes an immediate decrease in the [ATP]/[ADP_{free}] ratio which, unlike other transitory metabolic perturbations, persists with ongoing stimulation (Green et al. 1992). The imbalance between energy requirement and energy supply may represent an important signal triggering an appropriate adjustment in MHC isoform expression. According to the decreasing scale of tension costs, faster isoforms will be exchanged with slower isoforms.

It is presently unclear how alterations in the energy potential might affect gene expression in muscle because the putative relationship between the $[ATP]/[ADP_{free}]$ ratio and transcriptional and translational activities of genes encoding specific myofibrillar protein isoforms remains to be elucidated.

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