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Review

Human skeletal muscle fibres: molecular and functional diversity

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Abstract

Contractile and energetic properties of human skeletal muscle have been studied for many years in vivo in the body. It has been, however, difficult to identify the specific role of muscle fibres in modulating muscle performance. Recently it has become possible to dissect short segments of single human muscle fibres from biopsy samples and make them work in nearly physiologic conditions in vitro. At the same time, the development of molecular biology has provided a wealth of information on muscle proteins and their genes and new techniques have allowed analysis of the protein isoform composition of the same fibre segments used for functional studies. In this way the histological identification of three main human muscle fibre types (I, IIA and IIX, previously called IIB) has been followed by a precise description of molecular composition and functional and biochemical properties. It has become apparent that the expression of different protein isoforms and therefore the existence of distinct muscle fibre phenotypes is one of the main determinants of the muscle performance in vivo. The present review will first describe the mechanisms through which molecular diversity is generated and how fibre types can be identified on the basis of structural and functional characteristics. Then the molecular and functional diversity will be examined with regard to (1) the myofibrillar apparatus; (2) the sarcolemma and the sarcoplasmic reticulum; and (3) the metabolic systems devoted to producing ATP. The last section of the review will discuss the advantage that fibre diversity can offer in optimizing muscle contractile performance.

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1. Introduction

Skeletal muscle is a very heterogeneous tissue: heterogeneity is a property of all muscles and seems essential for their function. Skeletal muscles are employed to respond to a broad range of functional demands in each animal species and are even more different in different species. The functional flexibility which allows the same muscle to be used for various tasks from posture keeping, to repeated submaximal contractions and to fast and strong maximal contractions finds its basis: (1) in a powerful and accurate nervous control (motor neuron discharge rate and recruitment); and (2) in the availability in each muscle of numerous fibre types with distinct functional features (e.g.contraction time, peak power, maximum shortening velocity, resistance to fatigue).

Functional and structural heterogeneity of skeletal muscles has been the object of several recent reviews (Pette and Staron, 1990; Schiaffino and Reggiani, 1994, 1996; Moss et al., 1995). On the contrary, the aim of this review is restricted to the heterogeneity of human muscles.

The relevance of a review focussed on human muscle fibres goes beyond the interest of applied physiology and medical research. Other reasons can prompt interest for differentiation and specialization in human muscles. In the first place most of the information available on mammalian muscle fibres comes from small mammals whose size is 30 (rabbit) to 1000 (mouse) times smaller than man size. Although humans are not among the largest mammals their muscles have to cope with a limb length longer and a body weight certainly greater than those of small mammals. In addition, posture and locomotion of humans is peculiar as they stand and walk on their lower limbs and the upper limbs are free from any postural duty. This might

produce significant differences between lower limb and upper limb muscles. Finally, the development of nervous control of muscles acquires in humans peculiar features which are not comparable to any other animal species: it is not unlikely that this might also influence the functional requirements of muscle fibres which are generally satisfied by fibre diversity. For example, the asymmetric organization of human higher motor control centers is reflected in a different fatiguability and EMG activity in the left and right arms (DeLuca et al., 1986) and this is likely to imply differences in the muscle fibre level.

Several examples of the difference existing between human muscles and small mammal muscles have emerged in the last years. A *first* example can be given by the myosin heavy chain isoform IIB which is expressed in the muscles of small mammals such as mouse, rat, rabbit, but is not expressed in human muscles (Smerdu et al., 1994; Ennion et al., 1995). Interestingly, fibres containing MHC-IIB have been found also in the pig, a mammal which has a size similar to humans (Lefaucheur et al., 1998), but not in horse (Rivero et al., 1999). Apparently, the lack of this fibre type is not only a matter of body size. A *second* example can be represented by the less tight rules of isoform co-expression between myofibrillar protein: slow isoforms of MLC often coexist with fast MHC isoforms and vice versa, as shown by Larsson and Moss (1993). This would suggest a less tight control of gene expression in human muscle. Consistent with this is also the observation of the co-existence of actin skeletal and cardiac isoforms in human skeletal muscles (Gunning et al., 1983).

In this review, we will consider first how human fibre diversity is generated and how, nothwithstanding the large heterogeneity observed, human fibres can be grouped and fibre types identified. Then we will consider the molecular diversity and the functional diversity that derive from it. In more detail, we will consider diversity in mechanical and energetic properties, in excitability and excitation–contraction coupling, and in energy production systems and, at the same time, the molecular counterparts of such diversities. Finally, we will briefly speculate on how human muscle fibres with their functional heterogeneity can be used in vivo to optimize muscle performance.

2. Fibre types in human muscles

2.1. Generation of human fibre heterogeneity

As will be described in detail in the following sections of this review, the diversity among muscle fibres involves many aspects of muscle structure and function. The determination of virtually any functional parameter in a population of human muscle fibres shows large and continuous ranges of variability: examples are reported in Fig. 1. The generation of the diversity is based on gene regulation through two main mechanisms:

1. Qualitative mechanism: many muscle proteins exist in forms which are similar but not identical and which are called *isoforms*. Isoforms can derive from the same gene through alternative splicing or from different genes of the same family (isogenes). Replacement of isoforms represent a first mechanism to generate diversity among muscle fibres.
2. Quantitative mechanism: differential expression of the same gene. Many genes can be up-

and down-regulated independently of each other on the basis of factors such as neural discharge pattern, mechanical load, hormones etc. The proportion between the products of these genes will be therefore modified and new functional or structural features will appear.

The number of possible combinations generated by the two above-described mechanisms is, however, limited by constraints set by structural requirements or by rules of expression which define preferential associations between isoforms. For this reason the number of possible combinations tends to diminish and some more frequent phenotypes of muscle fibres appear. All attempts to classify muscle fibres in distinct fibre types aim to identify these more frequent phenotypes.

The best demonstration of the existence of specific fibre types is given by the conditions when fibre transformations take place, in the first place during development when fibre types emerge. Three waves of myoblast fusion occur in human muscles during prenatal development (Draeger et al., 1987). No difference among fibres is detectable until the 20th week. Later the adult phenotypes, as can be identified by ATPase staining and enzyme histochemistry (see below), appear and around one year postnatal all fibres are fully differentiated. Finally, during postnatal life the fibre phenotype can still be altered when due to variations in neural discharge patterns, mechanical load, hormonal stimulation, muscle fibre structural and functional features are changed.

In spite of the asynchrony produced by the different thresholds and turnover rates of the various muscle proteins, it is possible to observe that when muscle fibres undergo transformations, over a long period they move from one phenotype to the other (type transition). This implies the existence of rules which co-ordinate the expression of various genes and modulate various functions (energy production, energy consumption, calcium metabolism etc) in muscle fibres. Fibre type transformations have been studied in more detail in animals, particularly in small mammals such as rat or rabbit and they have been recently well reviewed by Pette and Staron (1997). Several data are, however, available also on muscle fibre plasticity in humans (for example induced by training or by disuse) and substantially confirm what is known from animal studies.

2.2. Various criteria for grouping muscle fibres: delineation of fibre types

Various classifications of fibre types have focused on one or on few structural or functional features and have defined groups of fibres on the basis of the selected parameter. Almost all classifications have been applied both to animal and human muscles.

Two different histochemical approaches have been utilized to classify fibre types. One classification is based on histochemical myofibrillar ATPase. By applying the assay for ATPase activity proposed by Padykula and Herman (1955), Engel (1962) achieved the first separation of type I from type II fibres. Later on the method was improved by Guth and Samaha (1969), Barnard et al. (1971) and Brooke and Kaiser (1970) applying the criteria based on alkali and acid preincubation. Subgroups of fibres were thus defined. In human trunk and limb muscles three main groups I, IIA and IIB and four intermediate groups IIC, IC, IIAC, IIAB (Staron and Hikida, 1992; Staron, 1997) can be delineated. Evidence in favour of a relation between

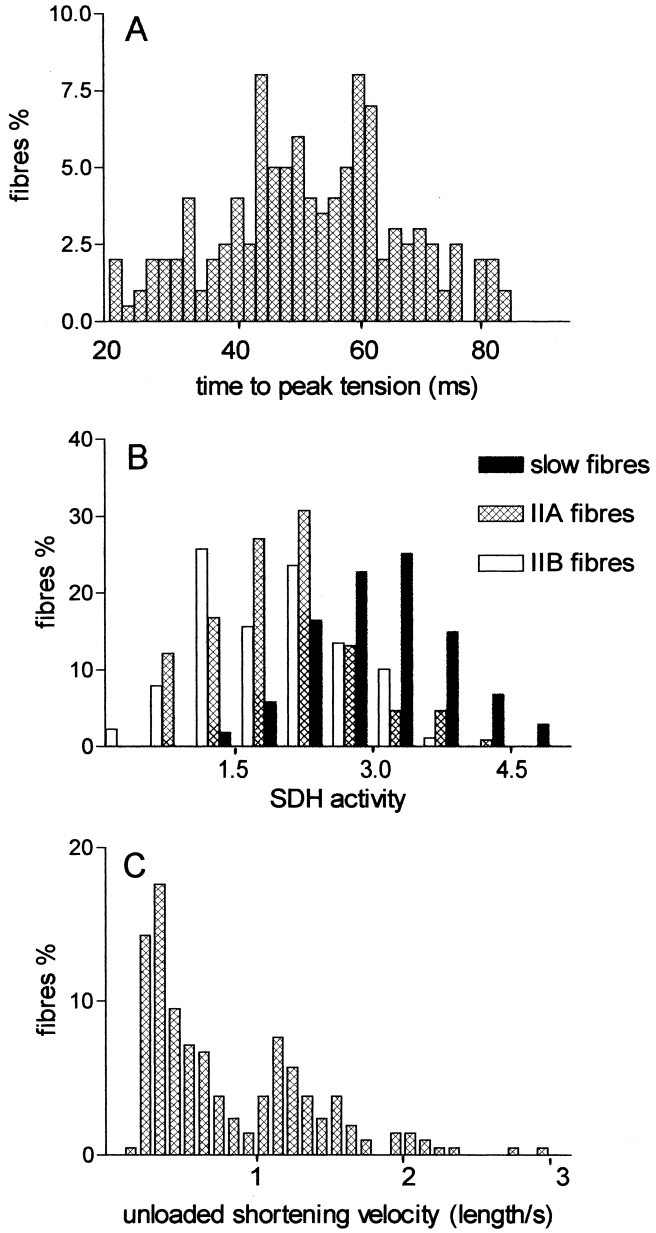

Fig. 1. Examples of heterogeneity of human muscle fibres. (A) Distribution of the contraction times, i.e. the times from stimulus to peak tension of fibre bundles in human biceps brachii, modified from Buchthal and Schmalbruch (1969). (B) Distribution of the SDH activity in single fibres from human tibialis anterior; fibres are divided into three groups according to their ATPase reactivity; enzyme activity is determined with a cytospectrophotometric method; modified from Reichman and Pette (1982). (C) Distribution of maximum shortening velocity of human single fibres from leg muscles (vastus lateralis, soleus and tibialis); three peaks are visible probably corresponding to slow, fast IIA and fast IIX fibres. Compilation of original data from R. Bottinelli.

myofibrillar ATPase and mechanical properties, in particular shortening velocity, has come from the work of Barany (1967) and Close (1972).

A parallel classification based on histochemical determination of metabolic enzymes was introduced by Ogata and Mori (1964) and by Padykula and Gauthier (1967). The types of fibres which are identified with these methods reflect the different content of mitochondrial enzymes (for example, SDH or COX) and can be therefore related with the oxidative potentials. The analysis of fibre types in motor units of identified functional properties (contraction time, fatigue-resistance, etc) revealed the relationship between fatiguability and metabolic properties (Burke et al., 1971; Kugelberg, 1973). Three fibre types were thus identified in skeletal muscles: SO fibres in slow (long contraction time), fatigue resistant motor units, FF fibres in fast (short contraction time), quickly fatigued motor units, FOG fibres in fast and more fatigue resistant motor units.

As discussed by Staron (1997) the assumption that classifications based on metabolic properties correlate with those based on myofibrillar ATPase is not justified. It is true that type I fibres are generally more oxidative than type IIB. Within each ATPase-based fibre type there is, however, a wide range of variations of metabolic enzyme activities (Hintz et al., 1984) and a large overlap has been found between fibres belonging to different types (Reichmann and Pette, 1982). Thus, attempts to combine the metabolic enzyme classification with the myofibrillar ATPase classification have been generally unsuccessful. For example, single fibre analysis of enzyme activities show a continuum distribution of aerobic-oxidative enzyme activities (as SDH, see Fig. 1) and of anaerobic enzyme activities (as LDH) regardless of the type defined by myofibrillar ATPase activity (Nemeth et al., 1981; Pette et al., 1981; Pette and Spamer, 1986).

Both classifications show further limitations as they apply only to fully differentiated adult muscle fibres and not to developing, degenerating or regenerating muscles. They also encounter difficulties when applied to different species or even to specialized muscles (e.g. masticatory or extraocular muscle) in each species. Up to eight different fibre types have been identified by ATPase staining in human masseter (Sciote et al., 1994). Human laryngeal muscles also need special treatments to classify fibres according to ATPase staining (Claassen and Werner, 1992). Histochemical ATPase staining of intrafusal fibres show a specific type (bag2) of fibres resistant to both alkali and acid preincubation in addition to alkali inactivated and acid resistant fibres (bag1) and to acid inactivated and alkali resistant fibres (chain) (Ovalle and Smith, 1972; Soukup, 1976).

More recently, electrophoretical (Fig. 2) and immunological identification of MHC isoform ((Danieli-Betto et al., 1986) and of human MHC isoform (Biral et al., 1988)) has allowed us to demonstrate that histochemical ATPase reactivity is based on the presence of specific MHC isoforms ((Staron and Pette, 1987), and for human muscles (Staron, 1991)). The combination of histochemical ATPase staining, antibody staining and electrophoresis has led us to recognize that four major fibre types are present in rat and rabbit fibres: type I or slow and three (IIA, IIB and IIX or IID) fast types (Bar and Pette, 1988; Schiaffino et al., 1989). Correlations between ATPase staining, antibodies against MHC isoforms and probes against their mRNAs (in situ hybridization) have more recently shown that human IIB fibres do not contain MHC-IIB but an isoform similar to the MHC-IIX found in rat and rabbit (Smerdu et al., 1994; Ennion et al., 1995).

Comparison between the results of morphometric analysis of sections stained for ATPase

activity and of electrophoretical-densitometrical evaluation of MHC isoforms confirms the general agreement between the two classifications in human muscles (Fry et al., 1994; Harridge et al., 1996; Berg et al., 1997), although histochemical misclassification of some fibers (especially in trained muscle) is possible.

In agreement with observations in small mammals, MHC isoforms are the main determinant

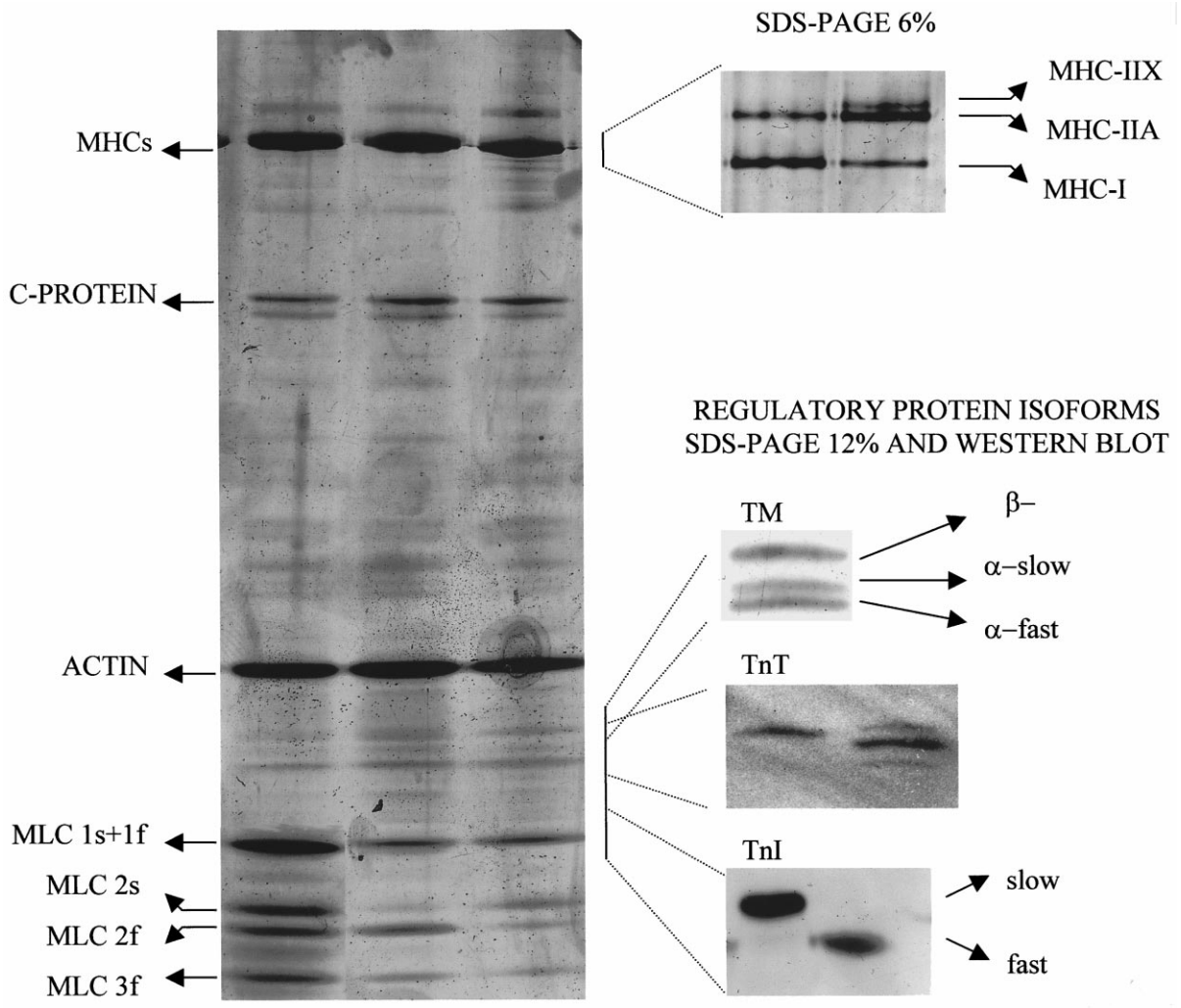

Fig. 2. Electrophoretic separation of proteins in single fibres of human muscles. On the left side three lanes of electrophoretical separation of single fibre protein on 12% polyacrylamide gel: lane 1 and lane 2, fast fibres, lane 3 slow fibre. On the right side details of single protein groups: from top to bottom, MHC isoforms separated on 6% polyacrylamide gel, tropomyosin isoforms, troponin T isoforms and troponin I isoforms on 12% gel followed by immunoblotting. Bands are identified by arrows. TnT bands are not identified by arrows for sake of clarity: a slow single fibres was loaded in the left lane which shows one band corresponding to Tn1 s; a fast fibre was loaded in the right lane that shows three bands corresponding from top to bottom to TnT1f, TnT2f and TnT3f. Courtesy of M.A. Pellegrino.

of several functional properties of human muscle fibres, such as maximum shortening velocity, ATPase activity, maximum power output and rate of tension redevelopment. This will be discussed in detail in a subsequent part of this review.

Furthermore, evidence is available that the expression of the isoforms of other myofibrillar proteins is co-ordinated with that of MHC isoforms: myosin light chain in the first place (Salviati et al., 1984; Jornstandt-Foegen et al., 1996), but also tropomyosin, components of the troponin complex (Salviati et al., 1984), Z line and M line proteins, sarcoplasmic reticulum ATPase.

On the basis of the above reasons MHC isoforms have been more and more often used as a molecular marker to identify fibre types. The classification based on MHC isoform would produce a completely reliable identification of fibre types (fibre typing) if the co-ordination was complete. There are however indications that co-ordination is not complete, particularly when fibres are in dynamic conditions and different thresholds for gene expression and different turnover times of proteins become evident.

In this review the MHC isoform composition will be adopted as the criteria for fibre classification. To be fully consistent with this choice the fibres containing MHC-IIX will be identified as IIX fibres, despite the fact that most papers have used in the past and still use now the term IIB fibres. No fibres containing MHC-IIB have been until now found in human muscles although the gene coding for this isoform is present in the human genome and has been localized on chromosome 17 (see below).

3. Molecular and functional heterogeneity of human muscle fibres

3.1. Heterogeneity of mechanical and energetic properties

3.1.1. Myofibrillar protein isoforms in human skeletal muscle fibres

Muscle fibres possess distinct mechanical and energetic properties. Sensitivity to activator calcium, mechanical power output, shortening velocity and rate of ATP hydrolysis greatly vary from fibre to fibre. The basis of this heterogeneity can be identified in the diversity of the molecular composition of myofibrils, the intracellular structure where the molecular motor of muscle contraction converts chemical into mechanical energy and where the regulatory mechanisms of the motor operate. Myofibrils are composed by sarcomeres arranged in series. The ordered structure of each sarcomere is based on the ordered alignment of two sets of filaments: thick filaments composed by myosin and myosin binding proteins and thin filaments composed by actin, nebulin and regulatory proteins (Fig. 3).

3.1.1.1. Myosin. Myosin is the most abundant muscle protein and plays the role of the molecular motor of muscle contraction. Sarcomeric myosins belong to class II myosin and are composed of two MHCs (mol. wt 200–220 kD) and four MLCs (mol. wt 20–16 kD), see Sellers et al. (1997) for a review of various myosin classes.

Eight genes of sarcomeric class II MHCs have been found in human genome (Weiss et al., 1999) (Table 1). Two of them are located in tandem on chromosome 14, whereas the other six are clustered on chromosome 17 (Weiss et al., 1999). Two genes code the isoforms *MHC-emb*

and *MHC-neo* which are expressed during development (Schiaffino et al., 1986b) and, in adult life, only during regeneration (Schiaffino et al., 1986a), in intrafusal muscles and in masticatory muscles (Butler-Brown et al., 1988; Stal et al., 1994). Three genes code for the isoforms, *MHC-I or β/slow, MHC-IIA, MHC-2X*, expressed in extrafusal fibres of limb and trunk skeletal muscles (Smerdu et al., 1994). The relative proportion of these isoforms varies from muscle to muscle (Klitgaard et al., 1990; Harridge et al., 1996). In lower limb muscles of adult healthy humans, MHC-I is the most abundant isoform followed by MHC-IIA. MHC-IIX is not expressed (for example in soleus) or little expressed (for example in vastus or tibialis anterior). In upper limb muscles the proportions of the fast MHC isoforms, MHC-IIA and MHC-IIX, is generally higher (Klitgaard et al., 1990; Harridge et al., 1996). In cardiac muscle MHC-I or β/slow is the dominant isoform in ventricular myocardium (Gorza et al., 1984), whereas *MHC-α* is expressed in atrial cardiac muscle (Gorza et al., 1984; Bouvagnet et al., 1989). *MHC-α* is also expressed in masticatory muscles and in a few slow fibres (Butler-Brown et al., 1988; Sciote et al., 1994). *MHC-IIB* gene has never been found expressed in human muscle fibres. Which MHC isoforms are expressed in human intrafusal fibres is not yet completely defined. Nuclear bag1 and nuclear bag2 fibers express predominantly MHC-I. In addition bag2 fibres contain MHC-neo. Nuclear chain fibers express MHC-emb, MHC-neo, MHC-α and other fast MHCs. The presence of an additional MHC isoform in nuclear bag fibres, which might correspond to *MHC-slow-tonic* present in non-mammalian vertebrates has been hypothesized (Eriksson et al., 1994; Pedrosa-Domellof and Thornell, 1994). In several mammalian species a MHC isoform with expression restricted to masticatory muscles, and therefore indicated as *MHC-m* or super-fast, has been identified. The human homologue gene has been localized in 7q22, but its expression in human muscles has not been demonstrated yet (Hoh et al., 1999).

Each of the two MHCs which form a myosin molecule combine with two MLCs, one alkali or DTNB extractable and one regulatory or phosphorylatable. Precise rules of co-expression dictate which MLC isoform combines with a given MHC isoform. In rabbit and rat skeletal muscles slow MHC associate with slow isoforms of regulatory (MLC2s) and alkali (MLC1sa or MLC1sb) MLC, whereas fast MHC isoforms associate with fast regulatory (MLC2f) and alkali (MLC1f or MLC3f) light chain isoforms (Salviati et al., 1982a; Wada and Pette, 1993). In human skeletal muscle preferential associations between MLC and MHC isoforms have been also found (Wada et al., 1990; Satta et al., 1997). When studied at single fibre level (Larsson and Moss, 1993) the co-ordination seems less strict: fast MLC isoforms are present in slow fibres and, vice versa, slow MLC isoforms appear in fast fibres.

Preferential associations are present also in immature muscles: MHC-emb is associated with MLC1-emb/a or MLC1f and MLC2f, whereas MHC-neo combines with MLC1f or MLC3f and MLC2f (Whalen et al., 1981).

The two fast alkali MLC isoforms (*MLC1f and MLC3f*) are produced by alternative splicing from the same gene (Peryasamy et al., 1984; Robert et al., 1984). The difference between the two isoforms is due to two different sites of transcription initiation: from one site exons 1 and 4 are expressed producing the MLC1f transcript, from the other site exons 2 and 3 are expressed producing MLC3f transcript. The two proteins are identical in the last 141 amino acids, but differ in the N-terminus: MLC1f has an additional N terminal sequence rich in alanine and proline, which is long enough to reach actin (Schaub et al., 1998). This odd sequence is probably responsible for its lower mobility in SDS-PAGE: MLC1f migrates as its

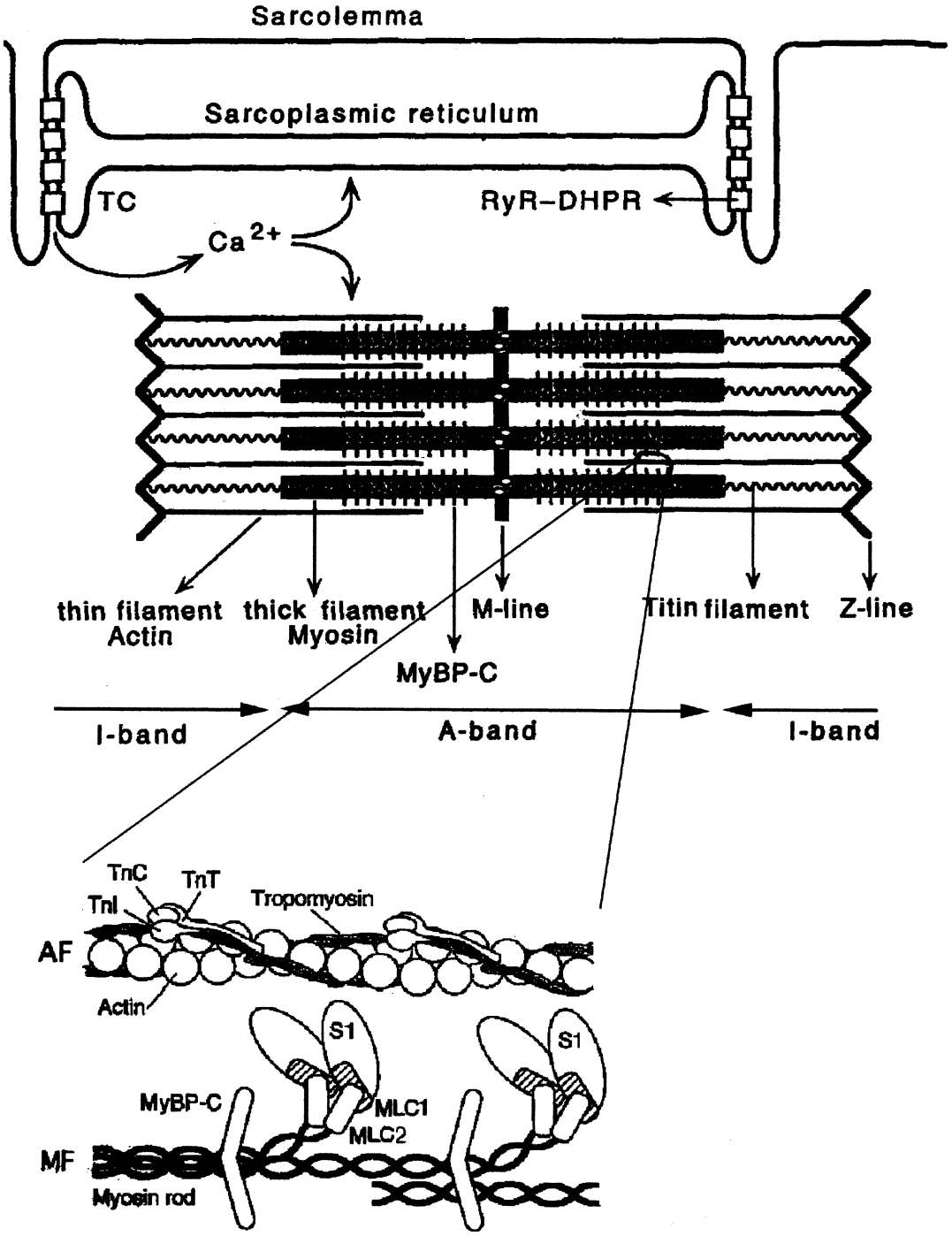

Fig. 3. Schematic drawing of the structures involved in the contractile response: from top to bottom sarcolemma with T tubules, sarcoplasmic reticulum and sarcomere with the thick and thin filament. A more detailed schema of thick and thin filament proteins is shown in the lower part of the figure [modified from Schaub et al. (1998)].

Table 1
Myofibrillar protein isoforms and their genes in human muscles. In some cases GenBank accession numbers are indicated in parentheses

Isoform	Pattern of expression	Gene	Locus	Reference
MHC-I/β-slow	slow fibres, ventricular myocardium	MYH7 (NM00257)	14q11.2-q13	Jaenicke et al. (1990)
MHC-α	atrial myocardium	MYH6	14q11.2-q13	Epp et al. (1993)
MHC-IIA	fast fibres	(AF111784)	17p13.1	Weiss et al. (1999)
MHC-IIX	fast fibres	(AF111785)	17p13.1	Weiss et al. (1999)
MHC-IIB	fast fibres (not found)	(AF111783)	17p13.1	Weiss et al. (1999)
MHC-emb	developing and regenerating muscles	HSMYHC (X13988)	17p13.1	Eller et al. (1989)
MHC-neo	developing and regenerating muscles	HSMYOHP (Y00821)	17p13.1	Karsch-Mizrachi et al. (1990)
MHC-exoc	extraocular muscles	MYH13 (AF111782)	17p13.1	Weiss et al. (1999)
MHC-m	masticatory muscles (not found)		7q22	Hoh et al. (1999)
MLC-2s/v	slow fibres, ventricular myocardium	MYL2	12q23-q24.3	Macera et al. (1992)
MLC-2f	fast fibres			
MLC-2m	masticatory muscles	MYL5	4p16.3	Collins et al. (1992)
MLC-2a	atrial myocardium			
MLC-1sa	slow fibres, non muscle cells			
MLC-1sb/sv	slow fibres, ventricular myocardium	MYL3	3p21.3-p21.2	Cohen-Haguenauer et al. (1989)
MLC-1/3f	fast fibres	MYL1	2q32.1	Cohen-Haguenauer et al. (1988)
MLC-1emb/a	developing muscles, atrial myocardium	MYL4	17q21	Cohen-Haguenauer et al. (1989)
Titin	all muscle fibres	TTN	2q31	Rossi et al. (1994)
MBP-C-cardiac	cardiac muscle	MYBPC3	11p11.2	Gautel et al. (1995)
MBP-C-slow	all muscle fibres	MYBPC1	11p11 or 12 ?	Weber et al. (1993)
MBP-C-fast	fast muscle fibres	MYBPC2	19	Weber et al. (1993)
Myomesin	all muscle fibres	MYOM1	18p11.31-p11.32	Speel et al. (1998)
M-protein	all muscle fibres			
H-protein	all muscle fibres	MYBPH	1q32.1	Vaughan et al. (1993)
Actin-α-card.	all muscle fibres	ACTC	15q13.2	Gunning et al. (1984)
Actin-α-skel	all muscle fibres	ACTA1	1p21	Gunning et al. (1984)
TM-α-fast	fast muscle fibres	TPM1	15q22	Eyre et al. (1995)
TM-α-slow	slow muscle fibres	TPM3	1p13-25	Laing et al. (1995)
TM-β	muscle fibres	TPM2	9p13.2	Tiso et al. (1997)
TnC-fast	fast muscle fibres	TNNC2	20q12-q13.11	Tiso et al. (1997)
TnC-slow/cardiac	slow muscle fibres and cardiac muscle	TNNC1	3	Schreier et al. (1990)

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Table 1 (*continued*)

Isoform	Pattern of expression	Gene	Locus	Reference
TnI-fast	fast muscle fibres	TNNI2	11p15.5	Tiso et al. (1997)
TnI-slow	slow muscle fibres	TNNI1	1q31.3	Tiso et al. (1997)
TnI-cardiac	cardiac muscle, immature skeletal muscle	TNNI3	19q13.4	Vallins et al. (1990)
TnT-slow	slow muscle fibres	TNNT1	19p13.4	Samson et al. (1992)
TnT-cardiac	cardiac muscle	TNNT2	1q32	Townsed et al. (1994)
TnT-fast	fast muscle fibres	TNNT3	11p15.5	Mao et al. (1996)
α-actinin 2	all muscle fibres	ACTN2	1q42-q43	Beggs et al. (1992)
α-actinin 3	fast muscle fibres	ACTN3	11q13-q14	Beggs et al. (1992)
Nebulin	all muscle fibres	NEB	2q24.1-q24.2	Rossi et al. (1994); Limongi et al. (1997)
Nebulette	cardiac muscle	NEBL	10p12	Millevoi et al. (1998)

molecular weight was 26 kD, instead of the real 21 kD. In rabbit and rat muscles the presence of the N-terminal extension has been considered responsible for the slower shortening speed found in muscle fibres rich in MLC1f (Sweeney et al., 1988; Bottinelli et al., 1994a).

One alkali slow MLC isoform (*MLC1sb*) is expressed in ventricular myocardium and in slow muscle fibres. A second alkali slow MLC isoform *MLC1sa* is only expressed in slow skeletal muscle fibres, in addition to smooth muscle and non-muscle cells (Salviati et al., 1984; Hailstones and Gunning, 1990). Its relative proportion is higher in human and rabbit muscles than in rat muscles and vary from muscle to muscle (Salviati et al., 1984).

A further isoform *MLC-1emb/a* is transiently expressed in fetal skeletal muscles and ventricular myocardium: in adult life its expression is restricted to atrial myocardium (Barton et al., 1988). It has been recently shown that the first 11 amino acids of human MLC-1emb/a can interact with the C terminus of actin (Timson and Trayer, 1997)

Two regulatory or phosphorylable MLC isoforms, *MLC2s and MLC2f*, are present in human skeletal muscle fibres (Salviati et al., 1984). MLC2s is expressed in myocardium and in slow muscle fibres and a second isoform MLC2s* is expressed in ventricular myocardium of human and other mammals (Price et al., 1980; Morano et al., 1988). The two varieties have the same molecular weight but different amino acid composition so that they can be separated only by 2D electrophoresis. Both varieties have one phosphorylation site for the calmodulin-dependent light chain kinase.

MLC2f is generally expressed in fast skeletal muscle fibres (Salviati et al., 1984). A third regulatory light chain MLC2a is expressed in atrial myocardium and has two sites for phosphorylation (Morano et al., 1989). A specific regulatory MLC isoform (MLC2 m) has been found expressed in feline masticatory muscles (Rowlerson et al., 1981). This isoform is coded by a gene very similar to the human MYL5 gene (Qin et al., 1994), however no demonstration of the protein in human masticatory muscles is presently available.

3.1.1.2. Myosin binding proteins. Thick filaments are composed beside myosin of other proteins which can bind myosin (myosin binding proteins or MBP) (Fig. 3).

C-protein or MBP-C (molecular weight ~140 kDa) binds myosin at regular intervals along

the thick filament. The C terminal region contains a binding site for the rod portion of myosin and for titin (Furst et al., 1992; Okagaki et al., 1993). A second binding site for myosin subfragment 2 (S2) exists in the N terminal region (Gruen and Gautel, 1999). Its functional role is not completely understood. Removal of MBP-C causes a decrease in calcium sensitivity as indicated by experimental removal of MBP-C (Hofmann et al., 1991). The development of an idiopathic cardiomyopathy in the presence of mutation of MBP-C is strong evidence in favour of an essential role in the contractile process (Watkins et al., 1995). The mutations responsible for the cardiomyopathy generally produce a truncated MBP-C, lacking the C terminal region (Watkins et al., 1995). Evidence that the binding of the N terminal region of MBP-C with S2 can modulate force development by cross bridge without altering their total number has been given by a recent study (Kunst et al., 2000).

Three variants of MBP-C (cardiac, slow and fast) are expressed in human sarcomeric muscles. The cardiac form is strictly restricted to myocardium, whereas the slow skeletal isoform is expressed virtually in all muscle fibres. A fast isoform is additionally expressed in fast fibres, where it coexists with the slow isoform (Gautel et al., 1998). The cardiac isoform has a specific feature which is absent in slow and fast isoforms: three sites for phosphorylation by protein kinase A are located close to the N terminus in the cardiac isoforms. It has been suggested that phosphorylation can control the binding of MBP-C to S2 and, in this way, can regulate cross bridge activity (Kunst et al., 2000).

Among myosin binding proteins *titin* has unique features. Due to its extremely large dimensions (molecular weight 3000 kD) and due to the presence of specific binding sequences, subsequent segments of titin from the N terminus to the C terminus can bind Z line proteins (actinin, telethonin), actin, myosin and M line proteins (Labeit and Kolmerer, 1995b). Thus, titin forms a connection between Z lines and thin filament on one side and M lines and thick filaments on the other side. The free region of titin in the I band is considered responsible for the largest component of passive tension in muscle fibres.

N-terminal titin immunoglobulin domains Z1 and Z2 interacts specifically with telethonin (Mues et al., 1998). The interaction with telethonin is phosphorylation dependent and probably important during myofibrillogenesis.

Titin has at least two binding sites for skeletal muscle-specific calpain, p94; one is at the N2-line region and the other is at the extreme C-terminus. Phosphorylation sites and a kinase domain are located in the C terminal region. The presence of the phosphorylation sites, of the kinase domain and of the binding sites for muscle-specific calpain makes it likely that the titin filament also plays a role in myofibrillar signal transduction pathways (Labeit et al., 1997).

Only one titin gene has been identified in the human genome (Labeit and Kolmerer, 1995b). From this gene several isoforms are produced by alternative splicing. Splicing occurs at the M line region and at the free region in the I band. Splicing in the M-line region generate titin isoforms (at least one cardiac and one skeletal) which are related with the variable M line structure (see below). Several variants can be generated by alternative splicing in the I band region (Labeit and Kolmerer, 1995b). The tandem-Ig, the N2 domain and the PEVK domain are involved in the splicing processes. Expression of isoforms which differ in the length of the repetitive Ig domains offers an explanation for diversity in resting length. Expression of muscle tissue-specific length variants of the PEVK region by alternative splicing might cause the differences in the resting tension-length curve between various striated muscle types (Labeit et

al., 1997). For example, in the rabbit a shorter titin isoform (T2) and a longer isoform (T1) have been found expressed by fast and slow muscle fibres, respectively, and have been correlated with higher and lower resting tension (Wang et al., 1991; Horowits, 1992). In human soleus and diaphragm a "long" isoform comprising additional 53 Ig domains, the N2A domain and a PVEK region 2174 amino acid long has been found (Labeit et al., 1997). A lower resting tension and a longer resting length should be expected in these muscles if compared to fast muscles.

Inter-individual diversity in the titin isoform distribution in leg muscles has been recently reported (Fry et al., 1997).

Two additional proteins able to bind myosin (in the light-meromyosin domain) and titin (in the C terminal 250 K region) are localized at the level of the M line: *myomesin and M protein*. These two proteins together with MM creatine kinase, myosin and titin form the complex structure of the M-line. Cloning of M-protein and myomesin (Vinkemeier et al., 1993) has revealed their similarity not only in molecular weight (165 kD for M protein and 162 kD for myomesin) but also in structure, as both proteins contain several immunoglobulin and fibronectin repeats. Whereas in some mammalian species M protein is detected in fast but not in slow muscle fibres (Thornell et al., 1990; Obermann et al., 1996), human slow fibres contain a M protein different in mobility from that present in fast fibres (Salviati et al., 1984). Myomesin, on the other hand, seems to be similarly expressed in slow and fast fibres (Obermann et al., 1996). Observations on human muscle cell differentiation in vitro suggest that myomesin plays a more important structural role than M protein in the M line assembly (van der Ven and Furst, 1997). Among intrafusal fibres, bag2 and chain fibres contain M protein and myomesin, whereas bag1 fibres contain only myomesin.

The presence of different isoforms of the M line proteins might explain the distinct ultrastructural features of M line in slow and fast fibres. Slow fibres have a wide M line with five strong M bridge lines, fast IIA fibres have three strong and two weak M bridge lines and finally fast IIX fibres have three strong and two very weak M bridge lines (Sjostrom and Squire, 1977).

Another myosin binding protein, called *H protein or MBP-H*, is located on both sides of the M line at a specific site of thick filament corresponding to the third stripe. The human H protein gene has been cloned (Vaughan et al., 1993) and its product seems to correspond to a 58.5 kD protein, containing four Ig and four fibronectin like domains. No isoforms have been identified and its function is unknown.

3.1.1.3. Thin filament proteins. Thin filaments are composed of double helical actin filaments. Two filaments composed of tropomyosin (TM) molecules, bound tail-to-head, lie in the groove between the two actin filaments. Each tropomyosin spans seven actin monomers and is associated with a troponin complex. Each troponin complex is composed of three subunits, TnT, TnI, TnC (Fig. 3). A single strand of the giant filamentous molecule nebulin is associated with the thin filament in skeletal muscle fibers.

Actin is the main component of the thin filament. In the monomeric form actin is a globular protein with a molecular mass of about 41 kD. As thin filament length in human skeletal muscle is 1.25–1.3 μm (Walker and Schrodt, 1973), approximately 460 actin monomers polymerize to form the two strands of each thin filament. Two isoforms of actin are expressed

in human skeletal muscle fibres: α-skeletal and α-cardiac (Gunning et al., 1983). The two isoforms which differ by only four amino acids are encoded by two different genes (Gunning et al., 1984).

Tropomyosin (TM) is an elongated protein composed by two subunits, each with an alpha helical structure (molecular weight 34–36 kD). The tropomyosin filaments which are formed by head to tail assembly of about 35 TM dimers contribute to thin filament stiffness (Kojima et al., 1994) and regulate actin–myosin interaction (Geeves and Lehrer, 1994). Four distinct tropomyosin genes are present in the human genome. Three of them are expressed in skeletal muscles: TPM1 which codes TM-α-fast, TPM3 which codes for TM-α-slow and TPM2 which codes for TM-β (Fig. 2). Co-ordinated expression of TM isoforms with MHC isoforms has been found in the rabbit and in the rat (Schachat et al., 1985; Danieli-Betto et al., 1990). In human muscles slow fibres express TM-α-slow and TM-β, whereas fast fibres generally express TM-α-fast along with TM-β (Bottinelli et al., 1998).

Mutations of TPM1 gene which cause Familial Hypertrophic Cardiomyopathy, have been shown to alter calcium sensitivity of skeletal muscle fibres (Bottinelli et al., 1998). Mutations of the TPM3 gene, encoding TM α-slow have been found to cause a dominant form of nemaline myopathy (Laing et al., 1995). It is hypothesized that the mutation strengthens the binding of tropomyosin to actin leading to the formation of the rod bodies, which are mainly composed by actin and actinin. The recessive form of nemaline myopathy is caused by mutations in skeletal α-actin (Nowak et al., 1999) and nebulin (Pelin et al., 1999).

Each subunit of the *troponin complex* [troponin C (Tn-C), troponin I (Tn-I) and troponin T (Tn-T)] is present in human skeletal muscles with two or more isoforms.

TnC is a calcium binding protein belonging to the same family as myosin light chains, calmodulin and parvalbumin. Two isoforms of troponin C are expressed in skeletal muscles: *TnC-fast* and *TnC-cardiac/slow*. The major difference between the two isoforms can be identified in the lack of a low-affinity calcium binding site: whereas TnC-fast exhibits two high affinity and two low affinity calcium binding sites, TnC-cardiac/slow shows only one low affinity site in addition to two high affinity sites. The regulatory function is based on binding calcium on the low affinity sites (Collins, 1991).

In rat muscles TnC-fast is expressed in fast muscle fibres in association with fast MHC isoforms, whereas TnC-cardiac/slow is expressed in fibres containing slow MHC (Danieli-Betto et al., 1990). Data on human single muscle fibres support a similar distribution also in human muscles (Salviati et al., 1984).

TnI is the second subunit of the troponin complex. Binding sites for TnC, TnT and actin give TnI the ability to interfere directly with actin-myosin interaction in relation with calcium binding to TnC. A slow and a fast isoform of troponin I are expressed in human skeletal muscle fibres (Fig. 2), the former being present in slow fibres and the latter in fast fibres (Salviati et al., 1984).

Troponin T (TnT) binds, in addition to TnC and TnI, also TM in two different regions. One of the two binding sites for TM (TnT2) is close to the binding sites for TnC and TnI and is calcium sensitive, whereas the other (TnT1) is calcium insensitive [see for recent reviews (Perry, 1998; Solaro and Rarick, 1998)]. The isoform pattern of troponin T is very complex: from three isogenes (TnT-fast, TnT-slow and TnT-cardiac) a large number of isoforms are generated by alternative splicing.

The *TnT-fast* gene can potentially produce up to 64 variants due to the alternative splicing of five exons at the 5′ end and one exon at the 3′ end. As shown by Briggs and Schachat (1996) only a limited number of splicing patterns occurs in mammalian muscles: this gives origin to a limited number of isoforms: six in fast rabbit muscles (EDL) and four to five in rat (Briggs and Schachat, 1996). These isoforms are identified with numbers increasing with decreasing molecular weight: TnT1f, TnT2f, TnT3f, TnT4f. Preferential associations between TnT1f and MHC-2X, TnT3f and MHC-IIA, TnT4f and MHC-IIB have been observed in rat single fibres (Galler et al., 1997). Electrophoretical and immunochemical studies have revealed the presence of four major variants of TnT fast in human limb skeletal muscles (Anderson et al., 1991; Sabry and Dhoot, 1991).

The *TnT-slow* gene can also produce some variants by alternative splicing of two exons. In rat and rabbit, two isoforms (TnT1 s and TnT2 s) have been identified in slow fibre (Schmitt and Pette, 1990). In human muscles isoforms derived from alternative splicing of TnT-slow have also been demonstrated (Gahlman et al., 1987; Samson et al., 1994).

Isoforms derived from *TnT cardiac* are expressed in atrial and ventricular myocardium. However, there are indications that two isoforms derived from TnT-cardiac are present in fetal skeletal muscles of rat (Saggin et al., 1990) and human (Anderson et al., 1991). Re-expression of cardiac TnT isoforms in skeletal muscles of patients affected by renal disease has been recently demonstrated (McLaurin et al., 1997).

Preliminary data on single fibres of human vastus lateralis identify four isoforms (TnT1 to TnT4 with increasing molecular weight) among which TnT1 and TnT3 are expressed in slow fibres and TnT2 is selectively localized in fast fibres (Pellegrino et al., 1999)

Two-dimensional electrophoresis additionally shows that each TnT isoform exists in several charge variants: these forms disappear upon treatment with phosphatase thus indicating that they are produced by multiple phosphorylation.

3.1.1.4. Nebulin. Nebulin (MW 900 kD) and nebulette (MW 107 kD) are, in spite of the different size, very similar proteins which share structure and function and can be therefore considered as isoforms. The C-terminal regions of nebulin and nebulette are identical in domain organization and are an integral part of the Z disc structure. Their N termini appear to project into the I-band and form a template for thin filament (Wang and Wright, 1988; Labeit and Kolmerer, 1995a; Millevoi et al., 1998). Expression of nebulette is restricted to cardiac muscle (Moncman and Wang, 1995) and expression of nebulin to skeletal muscles.

Most of the nebulin sequence (ca 97%) consists of 185 copies of a module of 35 residues. The central 154 copies are grouped into 22 super-repeats of seven-modules, each corresponding to 38.5 nm thin filament repeat. According to the model proposed by Pfuhl et al. (1996) nebulin super-repeats correspond to the 38.5 nm repeats of actin filament, each comprising seven actin monomers, one tropomyosin and one troponin complex. Repeats 2 to 7 of human nebulin, located in the N-terminal part of the super-repeat region of the nebulin molecule seem to be able to induce actin polymerization in vitro (Gonsior et al., 1998). These data support the idea that nebulin filaments are the template for thin filament organization.

In the region of interaction with the thin filament, multiple isoforms can be generated by alternative splicing. This might give origin to tissue-specific size variations of nebulin which have been found in vertebrate skeletal muscles. A correlation seems to exist between the size of

nebulin variants and the length of thin filaments in various muscles and animal species (Kruger et al., 1991). No information is presently available about nebulin heterogeneity in human muscles.

Recent evidence identifies nebulin as the gene responsible for the recessive form of nemalin myopathy (Pelin et al., 1997, 1999). This disease is characterized by the presence in the cytoplasm of muscle fibres of elongated structures, indicated as ''rod bodies'' which are aggregate of polymerized actin.

A nebulin-like protein, able to bind actin filament, indicated as *N-RAP*, has been recently localized at the myotendinous junction in skeletal muscle and at the intercalated disc in cardiac muscle. Based on its structural organization, actin binding properties, and tissue localization, N-RAP is considered to play a role in anchoring the terminal actin filaments of the myofibril to the membrane and in transmitting tension from the myofibrils to the extracellular matrix (Luo et al., 1997).

3.1.1.5. Z bands. In human muscles slow fibres have wider Z band than fast fibres, the fast IIX fibres having the thinner Z band (Sjostrom and Squire, 1977). Each Z-line consists of two sets of thin filaments anchored to each other by a network of transverse filaments. Each thin filament makes in this way connection with four thin filaments belonging to the adjacent sarcomere. Transverse filaments are mainly formed by actinin. Alpha actinins together with the N terminal domain of titin, and nebulin and several other proteins (tensin, Z protein, telethonin) are components of Z-line structure.

Alpha-actinins belong to a family of actin-binding proteins. Multiple isoforms of alpha-actinin are present in human tissues and are encoded by at least four distinct genes. Human skeletal muscle contains two sarcomeric isoforms, alpha-actinin-2 and -3. Alpha actinin-2 is expressed in all muscle fibres, whereas alpha actinin-3 expression is restricted to some IIA and all IIX fibres (Hoffman et al., 1989; Minetti et al., 1991). Alpha-actinins generally combine to form anti-parallel homodimers but formation of heterodimer between the two different isoforms has been reported (Chan et al., 1998).

3.1.2. Force–velocity properties and myosin isoforms

In vitro studies on human muscle fibres have developed very quickly since it has become clear that it was possible to dissect single muscle fibres, to be used for mechanical and biochemical determinations in vitro, from needle biopsy samples of human skeletal muscle. Skinned fibres, i.e. fibres whose plasma membrane is removed either chemically by detergents such as Triton X-100, or mechanically, have provided a reliable experimental model. Human skinned fibres proved to be very stable and reliable specimens, as good as skeletal muscle fibres of small mammals, which were the first to be used in this kind of study. As most of the techniques developed for analysis of skinned fibres from small mammals could be successfully employed for the analysis of human skinned fibres as well, in few years most of the main functional and biochemical properties of human fibres have been studied in detail.

Human skeletal muscle fibres, in keeping with what was previously observed in skeletal fibres of small mammals (Bottinelli et al., 1991), show a very large variability (7–10-fold) in force–velocity (FV) properties, as maximum shortening velocity or peak power. As a continuum distribution of all FV parameters can be observed, fibres can be found that shorten at any

velocity between the lowest and the highest values of the whole population (Figs. 1 and 4A) and this is true for all FV properties (Fig. 4B and C). When muscle fibres are grouped on the basis of their MHC isoform composition, clear differences among types are seen (Tables 2 and 3) for most FV properties, with the exception of tension (see below). However, a large variability in each property is seen within each fibre type.

3.1.2.1. Unloaded shortening velocity (V_o). Unloaded shortening velocity (V_o) is determined using the slack-test technique (Edman, 1979) that allows to directly determine V_o, although it does not give any information on the velocity of shortening at loads different from zero. As can be seen in Table 2, V_o of human fibres varies between: 0.26 l/s (type I) and 2.42 (type IIX) l/s at 12°C (Bottinelli et al., 1996) or 0.35–3.68 l/s (Larsson and Moss, 1993) at 15°C or 0.43–5.59 l/s (Widrick et al., 1996a) at 15°C. Therefore, V_o is consistently about ten times lower in slow than in IIX fibres, IIA fibres being intermediate, and hybrid fibres being intermediate between pure fibre types (Larsson and Moss, 1993; Bottinelli et al., 1996; Widrick et al., 1996a). Interestingly, in small mammals differences between the slowest, type I fibres, and the fastest, type IIB fibres, although still very clear, are somewhat less pronounced [3–4 times in the rat (Bottinelli et al., 1994a) and 4–5 times in the rabbit (Sweeney et al., 1988)]. In humans, V_o varies several fold among fast fibres of the same type (IIA and IIX fibres), whereas V_o variability among slow fibres is much less pronounced (Larsson and Moss, 1993; Bottinelli et al., 1996; Widrick et al., 1996a). Similar large variability in V_o among fast fibres of the same type has been shown in small mammals (Bottinelli et al., 1994a), and has been explained in the rat and in the rabbit on the basis of differences in alkali MLC isoform composition: the higher the content in MLC3f the higher the shortening velocity of fast fibres (Bottinelli et al., 1994a). No definitive explanation is available in humans for the variability in V_o among fibres of the same type. In human fibres, in fact, difficulties were found in relating V_o to a specific MLC expression pattern (Larsson and Moss, 1993; Widrick et al., 1996a, 1997a). This might be due to a less strict coordination of MHC and MLC expression in humans in comparison to small mammals, i.e. to the frequent coexpression of slow and fast isoforms of alkali and regulatory MLCs in the same human fibre. The majority of the available information point to some role for regulatory MLCs: the presence of MLC2s is seems to be associated with lower V_o values (Larsson and Moss, 1993; Larsson et al., 1997). No alkali MLC role has been found by Larsson and colleagues (Larsson and Moss, 1993; Larsson et al., 1997) and little if any impact has been shown by Widrick et al. (1996a). It cannot be ruled out that other factors might determine variations in V_o in fibres with the same MHC composition: (1) small amounts of a coexpressed MHC might not be detected and might somewhat affect V_o of fast fibres, i.e. IIA MHC coexpressed with IIX MHC could significantly slow down V_o of IIX fibres; (2) existence of undetected slow MHCs might determine variations in V_o among slow fibres.

Other issues related to V_o variability have raised interest. As regards the importance of the muscle of origin, it is unlikely that V_o of a given fibre type is consistently and significantly different according to the muscle of origin. The first work in which V_o of type I, IIA and IIX fibres of different human muscles of the same subjects were compared (vastus lateralis, soleus, triceps) suggested very similar V_o of the same fibre type regardless the muscle of origin (Harridge et al., 1996). On the contrary, V_o of gastrocnemius type I fibres was found significantly lower than V_o of soleus type I fibres by Widrick et al. (1997b). It cannot be ruled

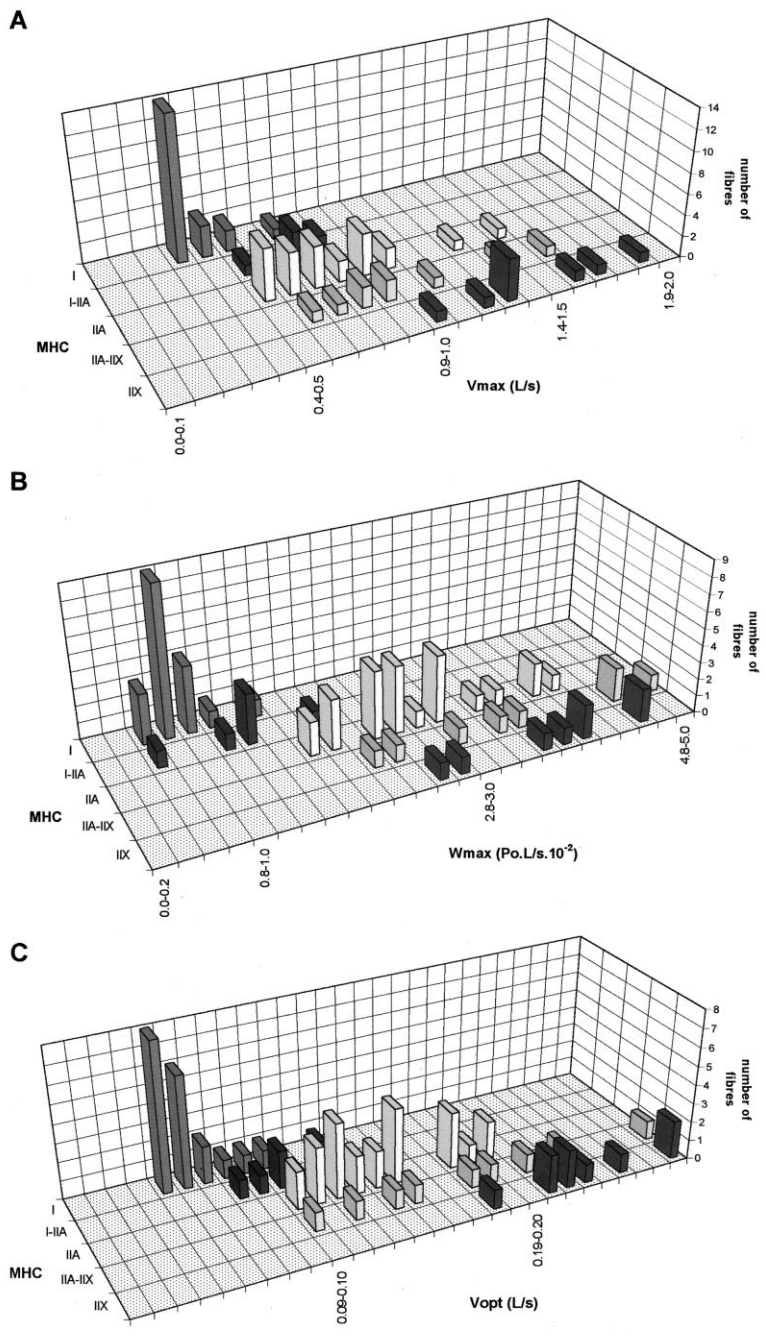

Fig. 4. Distribution of maximum shortening velocity (V_{max}) (panel A), maximum power (W_{max}) (panel B), and optimal velocity (V_{opt}) (panel C) in a population of 67 human skeletal muscle fibres. Fibres are grouped on the basis of their myosin heavy chain (MHC) isoform composition determined by polyacrylamide gel electrophoresis (SDS-PAGE). W_{max} is expressed in P_o l s^{-1}, i.e. is normalized over P_o (maximum isometric tension). Temperature, 12°C; sarcomere length, 2.5 μm. Data from Bottinelli et al. (1996).

out that V_o of a given fibre type changes in certain conditions according to the muscle of origin possibly based on shift in MLC composition. However, differences between type I, IIA and IIX fibres would be much larger than possible differences among fibres of the same type expressed in different muscles. Therefore, it is likely that shortening velocity of skeletal muscles in vivo mainly depends on fibre type composition and does not depend, or depends little, on possible fibre type-specific differences in shortening velocity based on the muscle of origin. It is unsettled whether exercise training can affect V_o of a given human muscle fibre type. V_o of type I, IIA and IIX fibres is not different before and after sprint training (Harridge et al., 1998). Endurance training does not affect V_o of type IIA and IIX fibres, but it does increase V_o of slow, type I, fibres (Widrick et al., 1996a). The underlying mechanism is not clear. Although a somewhat higher MLC3f content is observed in slow fibres after training, the MLC3 isoform shift observed cannot unequivocally account for the increase in V_o (Widrick et al., 1996a). It should also be considered that the same authors failed to show any difference in V_{max} (maximum shortening velocity determined from force–velocity curves) and maximum power (W_{max}) in another set of fibres obtained from the same subjects and studied in the same

Table 2
Values of maximum shortening velocity determined by slack test (V_o), maximum shortening velocity determined by extrapolation of force velocity curves (V_{max}), maximum power (W_{max}) and optimal velocity (V_{opt}) of human muscle fibres. All experiments were performed at or near slack length (2.5 μm SL). Unless specified data were obtained from fibres stored at −20°C in skinning solution + 50% glycerol

Reference	T°	V_o (l/s)			V_{max} (l/s)			W_{max} (W/l)			V_{opt} (l/s)		
		I	IIA	IIX	I	IIA	IIX	I	IIA	IIX	I	IIA	IIX
Larsson and Moss (1993)													
Glycerinated	15	0.35	1.07										
Freeze-dried		0.31	0.77	3.04									
Bottinelli et al. (1996)	12	0.26	1.12	2.42	0.32	0.72	1.29	0.35	1.63	3.03	0.05	0.14	0.22
Widrick et al. (1996a,b)													
Untrained	15	0.43	1.90	5.59	0.58	1.24	1.43	1.59	7.80	15.37			
Endurance trained		0.41	2.01	5.27	0.58	1.14		1.63	7.27				
Larsson et al. (1996)[a]													
Pre-bed rest	15	0.35	1.15										
Post-bed rest		0.18	1.19										
Larsson et al. (1997)													
Young	15	0.25	0.98										
Elderly		0.18	0.83	1.92									
Harridge et al. (1998)[b]													
Untrained	12	0.28	1.19										
Sprint trained		0.28	1.52										
Widrick et al. (1999)[c]													
Pre-flight	15	0.64	2.90		0.38			1.06	5.01		0.06		
Post-flight		0.83	4.49		0.55			1.00	4.65		0.08		

[a] Bed rest lasted 6 weeks.
[b] Data were obtained from frozen biopsy samples.
[c] Biopsy samples were taken pre and post 17 days spaceflight.

Table 3
Cross-sectional area (CSA), specific tension (P_o/CSA), isometric ATPase activity (ATPase), calcium sensitivity (pCa50%) and cooperativity (Hill slope) of human skeletal muscle fibres. Unless specified data were obtained from fibres stored at −20°C in skinning solution + 50% glycerol

Reference	T°	CSA (μm^2)			P_o/CSA (kN/m^2)			ATPase (mM ATP/s)			pCa50%			Hill slope		
		I	IIA	IIX	I	IIA	IIX	I	IIA	IIX	I	IIA	IIX	I	IIA	IIX
Fink et al. (1990)[a]	23				261	365	301				6.05	5.96	6.07	2.2	3.3	4.7
Ruff and Whittlesey (1991)[b]	22				217	227	224				5.86	5.60	5.61	1.74	2.28	2.53
Larsson and Moss (1993)																
Glycerinated	15	2370	2740	3410	210	200										
Freeze-dried		2480	2650	2650	120	120	190									
Lynch et al. (1994)[c]																
pH 7.1	22				292	397	352				6.16	6.09	6.11	2.02	2.99	3.79
pH 6.6					270	313	286				5.97	5.84	5.89	2.46	3.42	4.04
Bottinelli et al. (1996)	12	9278	7922	6294	44	61	62									
Stienen et al. (1996)	20				114	136	171	0.10	0.27	0.41						
Widrick et al. (1996a,b)																
Untrained	15	5150	5024	5407	143	156	170				6.04	5.91		2.11	3.01	
Endurance trained		4415	4298	6079	141	152	136				6.06	5.95		2.27	2.88	
Larsson et al. (1996)[d]																
Pre-bedrest	15				146	157										
Post-bedrest					83	92										
Larsson et al. (1997)																
Young	15	2820	2770		270	360										
Elderly		3090	3840		260	260	300									
Bottinelli et al. (1998)[e]																
WT TM	12	10127	10236		40	60					5.53	5.71		2.80	4.94	
Mutant TM		8879	6574		34	56					5.58	5.80		2.80	4.40	
Harridge et al. (1998)[f]																
Untrained	12	6296	5144		23	30										
Sprint trained		6531	5489		28	41										
Widrick et al. (1999)[g]																
Pre-flight	15	7234	10381		135	134					5.90			2.29		
Postflight		6079	7539		129	143										

[a] Fibres were used fresh, immediately after the biopsy.
[b] Fibres were classified in the original paper in SO, FOG and FG by histochemistry.
[c] Fibres were studied at pH 7.1 and pH 6.6.
[d] Bed rest lasted 6 weeks.
[e] Fibres from patients carrying a Asp175Asn mutation in α-fast tropomyosin (mutant TM) were compared with fibres from controls (WT TM).
[f] Fibres were obtained from frozen biopsies.
[g] Biopsy samples were taken pre and post 17 days spaceflight.

experimental conditions (Widrick et al., 1996b). Unloading/disuse have been shown to affect V_o of type I human fibres. Both after bed rest (Widrick et al., 1997a) and after 17 days spaceflight (Widrick et al., 1999), V_o of slow fibres from human soleus muscle was found to be higher than V_o of control slow fibres. The increase in V_o was attributed to an increase in myofilament lattice spacing induced by disuse. On the contrary, after 6 weeks bed rest V_o was found to be lower in slow fibres from vastus lateralis muscle than in control slow fibres consistently with an increase in MLC2s content (Larsson et al., 1996). Aging might also affect V_o of a muscle fibre type. In the only study to date in which properties of single muscle fibres from young and elderly subjects were compared, V_o of type I and IIA fibres was found to be lower in old than in young subjects (Larsson et al., 1997). Such decrease was not due to a shift in alkali MLC isoform expression and not clearly related to regulatory MLC content either (Larsson et al., 1997). On the contrary, in prior polio patients type I fibres from cronically overused motor units of tibialis anterior muscle have higher V_o than type I fibres from control, and this is accompanied by an increase in MLC2f relative content (Borg et al., 1995).

In conclusion V_o variability both in normal conditions and following muscle adaptations is mainly, but not completely explained on the basis of MHC isoform composition. The role of MHCs in determining V_o is supported also by evidence regarding Familial Hypertrophic Cardiomyopathy (FHC), a hereditary disease linked to point mutations in myofibrillar protein. The point mutations in MHC-I or β/slow are generally associated with a decrease in V_o of slow skeletal muscle fibres which contain this particular isoform (Lankford et al., 1995). The role of alkali and regulatory MLCs in determining V_o is still unsettled. Interestingly, the slow fibres, that in the same muscle have a low V_o variability compared to fast fibres, show significant differences in V_o when compared in different muscles and in different conditions [aging (Larsson et al., 1997), endurance training (Widrick et al., 1996a)]. This is interesting in light also of some evidence about the existence of more than one slow MHC isoform at least in rat (Hughes et al., 1993; Fauteck and Kandarian, 1995). Some of the subtle differences reported above, i.e. between type I fibres from gastrocnemius and soleus (Widrick et al., 1997b) or between type I fibres before and after endurance training (Widrick et al., 1996a), might be due to differences and changes in myofilament lattice spacing. Changes in myofilament lattice spacing, in fact, have been suggested as possible determinants of V_o differences before and after bed rest (Widrick et al., 1997a) or space flight (Widrick et al., 1999) and might play a similar role in other conditions as well.

V_o of human fibres shows a very striking dependence on temperature. The Q10 value of V_o for human fibres in the range 12–22°C is higher than 5.0 (Bottinelli et al., 1996) (Fig. 5). Such temperature dependence is unexpectedly high in comparison to previous determinations that suggested a Q10 of about 2.0 in whole skeletal muscles of the rat in the temperature range 10–35°C (Ranatunga, 1984), in intact frog muscle fibres between 0 and 12°C (Edman, 1979) and in rabbit skinned psoas fibres over the temperature range 5–30°C (Pate et al., 1994). However, a Q10 above 5.0 for human skeletal muscle fibres is consistent with a Q10 of 4.6 observed in cardiac trabeculae from rat (de Tombe and ter Keurs, 1990) and appears genuine, as supported by velocity measurements done independently using the load clamp technique (Bottinelli et al., 1996). The high Q10 for V_o in human muscle fibres might be explained by the different preparations used for Q10 determinations, by the different temperature sensitivity of rat, frog and humans, and by variations in temperature sensitivity depending on the temperature range

studied. Arrhenius plots, in which velocity is plotted versus the reciprocal of temperature, are not linear, but bi-phasic suggesting higher temperature sensitivity at lower temperature ranges. Indeed, Q10 values of 2.4–3.5 between 10 and 25°C and of 1.8–2.0 between 25 and 35°C have been reported in intact EDL and soleus muscles of the rat (Ranatunga, 1984), and Q10 values 1.7 times higher between 5 and 15°C than between 15 and 35°C have been shown in skinned fibres from rabbit psoas muscle (Pate et al., 1994). A continuous decrease in Q10 of actin sliding speed on myosin from rabbit skeletal muscle in in vitro motility assays has been shown

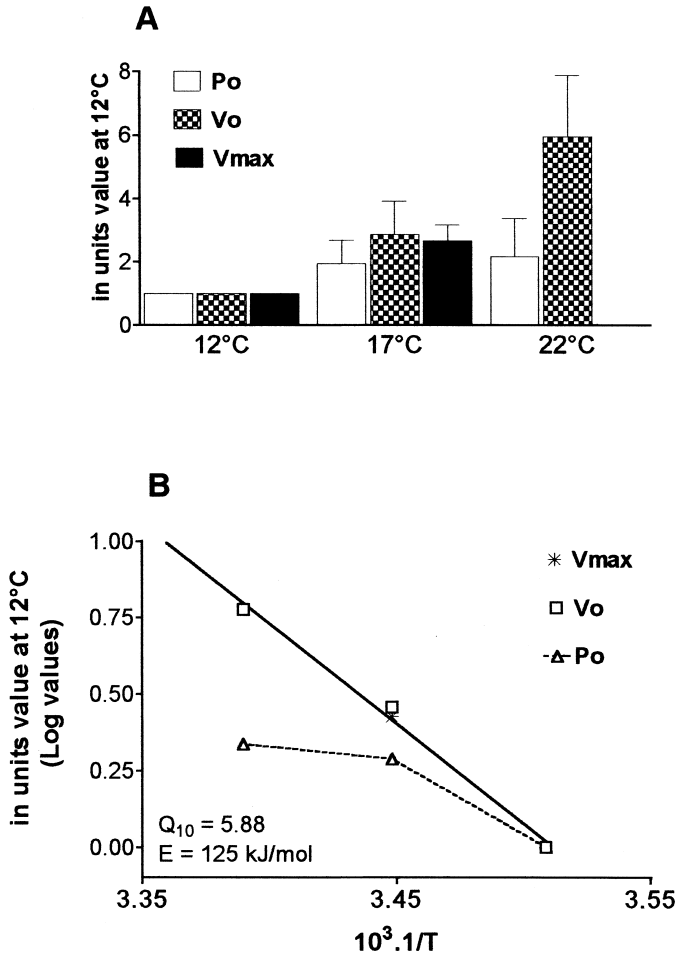

Fig. 5. Temperature dependence of tension (P_o), maximum shortening velocity determined by slack-test (V_o) and maximum shortening velocity determined from force–velocity relations (V_{max}) in the temperature range 12–22°C. (A) Each column represents the mean values of the ratios between each parameter at test temperature (17°C and 22°C) and at control temperature (12°C). Bars represent SD. V_{max} was not studied at 22°C. (B) Arrhenius plots for V_o, V_{max} and P_o. The logarithm of the mean values of the ratios between each parameter at test and control temperature are plotted versus temperature. SD are not shown for clarity. Temperature is expressed as $1/T\ 10^3$ where T is absolute temperature. The continuous line is the linear regression for V_o; the dotted line connect the values for P_o. Figure redrawn from Bottinelli et al. (1996).

from 3°C to 42°C (Anson, 1992). It might well be that temperature sensitivity is less in amphibians than in small mammals (Bennett, 1984) and in small mammals than in humans. It is still unsettled whether in humans, as in small mammals (Ranatunga, 1982, 1984), V_o of slow fibres is more temperature sensitive than V_o of fast fibres (Bottinelli et al., 1996).

Skeletal MLC2 can be phosphorylated as cardiac and smooth MLC2. However, at variance with P_o, there is no evidence of a modulatory role of MLC2 phosphorylation on V_o.

3.1.2.2. Isometric tension (P_o/CSA) and rate of tension rise. Specific tension (P_o/CSA) developed by human muscle fibres in vitro is highly variable, i.e. differences between the weakest and the strongest fibres are very large (10-fold) as shown in Table 3. Such large variability is little dependent on MHC isoform composition. P_o/CSA of slow fibres has been shown to be mostly (Bottinelli et al., 1996; Widrick et al., 1996a), but not invariably (Larsson and Moss, 1993) lower than P_o/CSA of IIA and IIX fibres. Among fast fibres differences in P_o/CSA are even less clear: no difference was found by Bottinelli et al. (1996) and by Larsson and Moss (1993); a significant difference was found by Widrick et al. (1996a). That P_o/CSA of human fibres is less affected by molecular composition than most other FV parameters is consistent with what observed in small mammals (Bottinelli et al., 1994a).

P_o/CSA values reported by different research groups vary rather widely between: 114 kN/m^2 and 171 kN/m^2 (Stienen et al., 1996) at 20°C, 100 kN/m^2 and 200 kN/m^2 (Larsson and Moss, 1993) at 15°C, 140 kN/m^2 and 170 kN/m^2 (Widrick et al., 1996a) at 15°C, 40 kN/m^2 and 60 kN/m^2 (Bottinelli et al., 1996) at 12°C. This is largely, but probably not only, due to the different approaches used in calculating CSA and to the different experimental conditions. On the basis of the observation that skinned fibres swell after removal of the plasma membrane (Godt and Maughan, 1977), CSA has been corrected by 20% by some authors (Larsson and Moss, 1993) and P_o/CSA consequently increased. In most works CSA is calculated assuming a circular shape of the fibres (Bottinelli et al., 1996; Stienen et al., 1996; Widrick et al., 1996a), whereas in some work width and depth of the fibres are determined and CSA is calculated assuming an elliptical shape (Larsson and Moss, 1993). Therefore, the reported CSA values of fibres used for P_o/CSA determinations vary from about 1800–2800 μm^2 (Larsson and Moss, 1993) to 5000 μm^2 (Widrick et al., 1996a) to 5000–9000 μm^2 (Bottinelli et al., 1996). CSA of human fibres determined on cross sections of muscle samples by histochemistry, using a fixation procedure that probably determines a shrinkage of muscle fibres, range from 2110 μm^2 to 11000 μm^2 in different muscles (Saltin and Gollnick, 1983), and is therefore more close to the largest CSA observed in isolated fibres. Temperature in the range 0–22°C strongly affects P_o (Bottinelli et al., 1996; Stienen et al., 1996) (see below). Not only ionic strength, but also the kind of salt (KCl versus K propionate) used to determine a given ionic strength affect P_o (Andrews et al., 1991; Godt et al., 1993). Phosphate concentration also strongly affects P_o, isometric tension decreases linearly with the logarithm of Pi concentration (Pate et al., 1998). Temperature, ionic strength, phosphate and composition of solutions are not the same in all works on the matter and might well determine to a large extent discrepancies in P_o/CSA measurements.

However, the large differences in the P_o/CSA values reported by different groups might be due not only to the different approaches used in determining CSA and to the different experimental conditions, but to some other determinants. Normalization of P_o over CSA is

heavily dependent on the assumption that myofibrillar protein content is a constant percentage of the fibre volume in all fibre types, in all muscles and in all conditions. Studies on muscle fibres from unloaded/disused muscles have suggested that, following atrophy, CSA of muscle fibres decreased less than myofibrillar protein content, i.e. variations of CSA and myofibrillar protein content might not be strictly dependent (Larsson et al., 1996; Widrick et al., 1997a, 1999). Should this be the case, normalization of P_o over CSA would fall short of its goal to give a precise idea of the amount of force developed by a given amount of myosin. A reliable normalization of force could be based on determination of myofibrillar protein density by electron-microscopy or quantitative electrophoresis. So far, electron-microscopy has been employed with this purpose only in frog, and has shown that frog heart and skeletal muscle develop very similar force, once the density of myofibrils in the two specimen is taken into account (Colomo et al., 1997). However, recently evidences have been reported that thick filament density might remain constant, whereas thin filament density decreases (Widrick et al., 1997a, 1999). A detailed analysis of sarcomere structure and myofilament lattice structure and spacing might be necessary to fully clarify the issue.

In conclusion, P_o/CSA, together with thermodynamic efficiency (see below), is the contractile parameter least affected by MHC composition. It is the parameter that varies more widely in different works, probably because its determination is exposed to more uncertainties than other parameters. Significant improvement can be achieved in studying P_o/CSA by a more precise determination of CSA and by normalization of force over myofibrillar protein density. It cannot be ruled out that P_o/CSA is also more variable from subject to subject than other parameters. However any further consideration requires a more precise determination of P_o/CSA in human muscle fibres.

P_o/CSA variability has been studied in relation to aging, physical training and disuse/unloading. Both type I and IIA fibres from elderly subjects have lower P_o/CSA than corresponding fibre types from young subjects; no such difference in P_o/CSA has been seen for IIA-IIX and IIX fibres (Larsson et al., 1997). Whereas there is evidence that in the mouse the age-induced decrease in P_o/CSA (Brooks and Faulkner, 1988; Phillips et al., 1993) might be due to myofibril loss and increase of inter-myofibrillar spaces (Ansved and Larsson, 1990), no such analysis has been so far performed in humans. The reason for the decrease in P_o/CSA of single fibres from elderly subjects is still unknown. Both sprint (Harridge et al., 1998) and endurance (Widrick et al., 1996a) training do not clearly affect P_o/CSA of individual human muscle fibres. Contrasting evidence is available on P_o/CSA of human muscle fibres after disuse/unloading. In one study (Larsson et al., 1996), P_o/CSA has been shown to decrease after 6 weeks of bed rest in vastus lateralis fibres and such decrease has been related to a decrease in myofibrillar protein content. In two studies by the same research group, P_o/CSA of soleus fibres was found not to be decreased after 17 days of bed rest (Widrick et al., 1997a), but significantly decreased after 17 days spaceflight (Widrick et al., 1999) in three of the four subjects studied. A decrease in thin, but not thick filament density was observed in both cases. More work is needed to clarify the impact of unloading on P_o/CSA and its mechanisms. However, the study of P_o/CSA of muscle fibres from unloaded/disused atrophic muscles has already produced the interesting outcome of prompting studies aimed to clarify whether CSA and myofibrillar protein synthesis change in parallel in all conditions. This is an interesting

issue to further the understanding not only of the differences in P_o/CSA, but also of the regulation of myofibrillar protein synthesis.

MLC2 phosphorylation represents an important regulatory mechanism, not only in smooth muscle but also in cardiac and skeletal muscle (Sweeney et al., 1993). MLC2 phosphorylation, being activated by Ca^{++} through calmodulin, has been shown to occur during voluntary contractions in intact human muscle (Houston et al., 1985; Houston and Grange, 1991) and to determine potentiation of a twitch (Houston et al., 1985) and of electrically stimulated contractions at low frequency (Houston and Grange, 1991). The physiological role of such phenomenon is still unsettled. It has been suggested that, during maximum voluntary contractions, force enhancement following MLC2 phosphorylation could counteract the decrease in force due to fatigue (Houston and Grange, 1991). However, such a mechanism is bound to work only at low level of activation as that achieved during a twitch or during low frequency stimulation. The above findings in humans are consistent with the observation that in rabbit skinned muscle fibres MLC2 phosphorylation determines an increase in calcium sensitivity and therefore of force at submaximal Ca^{++} levels (Sweeney et al., 1993). As no impact has been observed at maximal Ca^{++} levels, it is very unlikely that MLC2 phosphorylation has any role in determining the large variability in the P_o/CSA values reported above which were obtained in maximally activated conditions.

In keeping with what is observed in small mammals and amphibians, P_o of human fibres increases with temperature. Interestingly, in human fibres, P_o increases almost 2-fold between 12°C and 17°C, but increases very little between 17°C and 30°C (Bottinelli et al., 1996; Stienen et al., 1996) (Fig. 5). As can be seen in Table 3, values of P_o/CSA obtained at room temperature (20–23°C) are consistently higher than values measured at 12–15°C. Such findings are in broad agreement with an in vivo determination of temperature sensitivity of force in evoked twitch contractions of first dorsal interosseus muscle (Ranatunga et al., 1987). As for V_o, little can be said about the possible different temperature sensitivity of type I, IIA and IIX human fibres. In small mammals it seems well established that slow muscles are more temperature sensitive than fast muscles (Ranatunga, 1982, 1984).

When skinned fibres are activated, the rate of tension rise depends not only on the actual rate at which contractile proteins develop force, but also on the rate of diffusion of Ca^{++} inside the myofibrils. To determine the actual rate of force development by the contractile material it is necessary to let force of skinned fibres to develop fully and then to release the fibre at zero load and re-stretch it to the initial length before force starts redeveloping (Brenner and Eisenberg, 1986; Metzger and Moss, 1990). The rate of force redevelopment can therefore be studied at the initial length in conditions of full activation. It should also be remembered that during force redevelopment after unloading complex intersarcomere dynamics occur, i.e. sarcomeres do not redevelop force under true isometric conditions. This has prompted an experimental approach in which sarcomere length is clamped, i.e. kept constant during the phase of force redevelopment (Metzger and Moss, 1990). The only available information on the rate of force redeveloping in human fibres has been collected just measuring the rate at which force recovered after a slack-test (Harridge et al., 1996). Consistent with what was observed in the rat (Metzger and Moss, 1990), the results show that type I fibres redevelop force much slower (10-fold) than IIX fibres and IIA fibres are intermediate. Being aware of limitations that might come from sarcomere length non-uniformities, it can be suggested that

although isometric force is little different in the different fibre types, the rate at which isometric force is built up largely varies according to MHC composition. Also for the rate of tension rise, however, variability is very high among fibres of the same type.

Clear differences between type I, IIA and IIX human muscle fibres have been shown also for the so called "stretch activation" or "delayed tension transient" (DTT). Quick (completed in less than 0.5 ms) and small (2–3% SL) stretches of maximally activated muscle fibres cause an immediate rise in force, followed by a decrease and finally by a delayed transient increase in force, generally called DTT. Human muscle fibres, consistent with what is observed in small mammals, show remarkable differences in the kinetics of DTT with type I fibres having the slowest and IIX fibres the fastest kinetics, IIA fibres being intermediate (Hilber and Galler, 1997). The mechanism underlying DTT is still unclear and, therefore, the meaning of such differences is difficult to understand. It has been suggested that stretch activation might be related to the kinetics of the power strokes of myosin heads (Galler et al., 1994; Hilber and Galler, 1997).

3.1.2.3. Maximum power (W_{max}) and optimal velocity (V_{opt}). Isolated single fibres can be kept maximally activated at low temperature (12–15°C) and allowed to shorten against constant and controlled loads. Force–velocity curves can be obtained and fitted by Hill hyperbolic equation $[(P + a)(V + b) = a(V_o + b) = b(P_o + a)]$ (Fig. 6). From the parameters of Hill's equation the intercept with the velocity axis, i.e. velocity at zero load or V_{max}, the curvature of the force–velocity curve, a/P_o, and the intercept with the force axis, P_o^* can be calculated. From the parameters of Hill's equation it is also possible to draw power-force and power-velocity curves and to calculate maximum power (W_{max}) and optimal velocity (V_{opt}), i.e. the velocity at which W_{max} is developed.

V_{max} is markedly lower (4-fold) in type I than in type IIX fibres, IIA fibres being intermediate, and shows a very large variability among fibres belonging to the same type (Bottinelli et al., 1996; Widrick et al., 1996b). Qualitatively V_{max} determinations are in agreement with V_o results and confirm that MHCs are the main, although not the only determinant of maximum shortening velocity. Large and still unexplained variability among fibres belonging to the same type is present for V_{max} as for V_o. It must be pointed out that in human skinned fibres, as in skinned fibres from small mammals, V_{max} and V_o values are not the same: V_{max} is consistently lower than V_o (Bottinelli et al., 1996). The reason for such difference is unclear. V_o measurements might be influenced by a delayed (viscous) component of series compliance (Seow and Ford, 1992), whereas V_{max}, being obtained by extrapolation of the force–velocity curve, might be significantly affected by even small errors or deviations from the hyperbolic shape in the low load region of the force–velocity curve (Julian et al., 1986; Bottinelli et al., 1991). Both determinations, therefore, bear some uncertainties that are probably responsible for the differences between V_o and V_{max}. In this regard, it is interesting to note that V_{max}/V_o ratio is different for different fibre types and greater when curvature is higher as in type I fibres (Bottinelli et al., 1996). V_o and V_{max} are very similar when intact frog fibres are used and an experimental approach especially designed to optimize measurements of both V_{max} and V_o is employed (Julian et al., 1986). There is general agreement that a unique maximum shortening velocity exists in single fibres, and that discrepancies are due to inaccuracies in V_{max} and V_o determinations.

Type I fibres have higher curvature of the force–velocity curve than IIA and IIX fibres (Bottinelli et al., 1996; Widrick et al., 1996b). This is also in agreement with what is observed in small mammals (Bottinelli et al., 1991) and is one of the determinants of the lower power output of type I fibres (see below). It should be noted that the curvature of the force–velocity curve is consistently higher in skinned fibres than in intact fibres (Julian et al., 1986). The observation that osmotic compression of the myofilament lattice in skinned fibres decreases the curvature of the FV relation (Goldman, 1987) suggests that the high curvature of skinned fibres might be due to the well known swelling of skinned fibres which follows membrane removal (Godt and Maughan, 1977) and causes an increase in myofilament lattice spacing. P_o^*, the value of isometric force obtained by extrapolation of the force velocity curve to zero velocity, i.e. the isometric force "predicted" by the force–velocity curve, is very similar in human fibres to P_o determined experimentally. This indicates a nearly perfect hyperbolic shape of the FV curve at high loads. In intact frog fibres P_o^* is higher (Edman, 1988; Edman et al., 1997) than P_o, and the FV relation is not perfectly hyperbolic at high loads. In rat skinned fibres, P_o^* was found to be lower than P_o by Bottinelli et al. (1991) and higher than P_o by Lou and Sun (1993). Although it was suggested that discrepancies among skinned fibre experiments and between skinned and intact fibre experiments can be due to differences in ionic strength of the solutions (Lou and Sun, 1993), the reason for such differences is still not definitely settled.

Among the parameters derived from the FV curve, W_{max} and the velocity at which W_{max} is

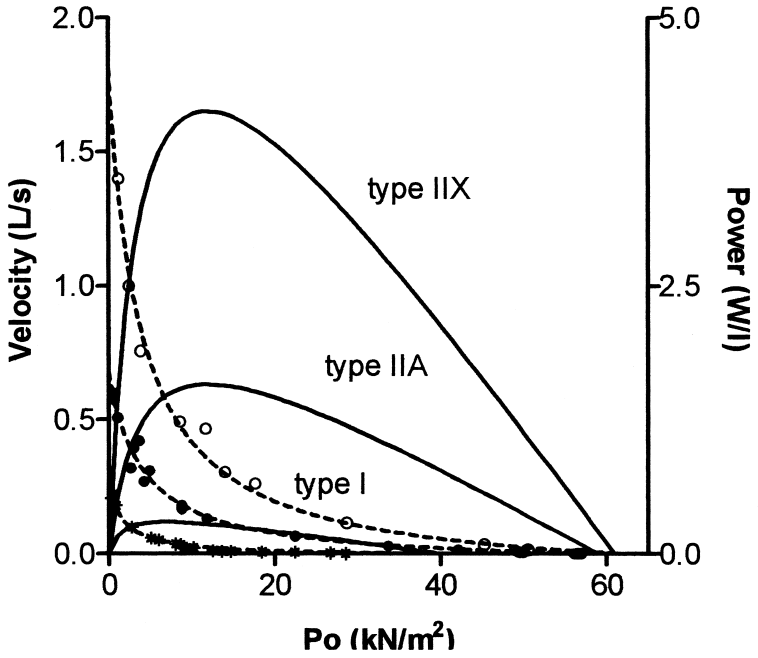

Fig. 6. Force–velocity curves (dotted lines) and power-force curves (solid lines) of representative type I (stars), type IIA (full circles) and type IIX (open circles) human skeletal muscle fibres. Force–velocity curves are fitted by Hill's hyperbolic equation and power-force curves are calculated from the parameters of Hill's equation. Power is in watt per liter (W/l). Temperature, 12°C; sarcomere length, 2.5 μm. Data from Bottinelli et al. (1996).

developed, V_{opt}, are possibly the most relevant to assess the role of the different human fibre types in modulating and optimizing human muscle performance in vivo. It is generally believed, in fact, based on experiments on fish and frog (Rome et al., 1988; Lutz and Rome, 1994), that muscles during normal movements in vivo shorten at a velocity at which their muscle fibres develop W_{max}. W_{max} of type I fibres is about 10-fold lower than that of IIX fibres, IIA fibres being intermediate (Fig. 6 and Table 2) (Bottinelli et al., 1996; Widrick et al., 1996b). The usual large and unexplained variability present among fibres expressing the same MHC isoform observed for V_o, V_{max}, P_o is seen also for W_{max} (Bottinelli et al., 1996). MLC isoforms do not affect W_{max} in rat fibres (Bottinelli and Reggiani, 1995) and are unlikely to play a role in modulating W_{max} in human fibres as they do not clearly affect V_o (Larsson and Moss, 1993). Power-force (Fig. 6) and power-velocity (Fig. 11) curves respectively show that type I fibres develop W_{max} at a lower load (2-fold difference), and at a much lower V_{opt} (4-fold difference), than type IIX fibres, IIA fibres being intermediate. Interestingly (Fig. 4C) the differences in V_{opt} among different fibre types are much more clear cut than differences in any other contractile or biochemical property; the variability in V_{opt} among fibres belonging to the same type is lower than for all other properties; the ranges of variability of V_{opt} for type I, IIA and IIX fibres hardly overlap, only hybrid fibres "bridge" the ranges of variability of pure fibre types. Therefore V_{opt} appears the most distinctive property of a fibre type. This is a relevant point. It suggests that in vivo for a given range of velocity of shortening there is only one fibre type available to produce W_{max} and therefore to optimize power production. V_{opt} distribution in human muscle fibre types suggests a possible way of optimization of muscle performance in vivo (see below).

3.1.3. Energetic properties and myosin isoforms

3.1.3.1. ATP consumption in isometric conditions and tension cost. Energetic studies performed on whole muscle and single fibres obtained from amphibians and small mammals (rabbit and rat), together with biochemical studies on purified myosin and myofibrils, have provided a rather complete picture of ATP hydrolysis by actomyosin interaction under steady state conditions. Differences between isometric and isotonic conditions, effects of sarcomere length, temperature, and pH have been described (Fenn, 1923; Woledge et al., 1985; Cooke et al., 1988; Stephenson et al., 1989; Potma et al., 1994a,b; Zhao and Kawai, 1994). Analysis of the enzymatic function of myosin subfragment 1 (S1) has demonstrated that the heavy chain component is the main modulator of ATPase activity (Wagner, 1981; Lowey et al., 1993). The role played by myosin isoforms in modulating ATPase activity has also been studied by comparing ATPase activity of myosin purified from slow and fast muscles in solution (Barany, 1967), and by histochemical analysis of cross-sections of slow and fast fibres (Mabuchi and Sreter, 1980a,b). Recently, techniques have been developed that allow to detect the small amount of ATP consumed by an isolated single fibre (Stienen et al., 1990; He et al., 1997), and the ATPase activity of different fibre types from rat has been studied (Bottinelli et al., 1994b). On the contrary, little information is available on human muscle fibres.

The only detailed study in which ATPase activity of isolated human muscle fibres has been determined so far shows that ATPase activity (at 12°C) varies very widely among muscle fibres from 0.044 mM ATP s^{-1} (type I) to 0.123 mM ATP s^{-1} (type IIX) (Stienen et al., 1996)

(Fig. 7A). Therefore, in isometric conditions, type I fibres splits ATP three times slower than type IIX fibres, type IIA fibres being intermediate, and hybrid fibres intermediate between pure fibre types. Such result is in agreement with previous observations on rat single muscle fibres (Bottinelli et al., 1994b). There are no ATPase activity determinations of human actomyosin in solution to use for comparison. Based on the above-reported values of ATP consumption rate in vitro and on the reported Q10 of 2.6, ATP consumption of human muscle fibres of different types in vivo has been calculated (Sahlin et al., 1998). The results (6.5 mmol/kg dw/s for slow fibres, 17.6 mmol/kg dw/s for IIA fibres and 26.6 mmol/kg dw/s for IIX fibres) are in very good agreement with in vivo estimates of ATP consumption rate (6.1 mmol/kg dw/s for slow fibres and of 12 mmol/kg dw/s for fast fibres) based on measurements of ATP, phosphocreatine and glycogen content of human muscle biopsies before and after maximal isometric contractions (Greenhaff et al., 1993) (see Section 3.3 for more details).

The ATPase activity varies 2–3-fold among fibres with the same MHC composition and therefore belonging to the same type. The molecular basis of such variability within each fibre type, especially fast types, is still unknown. MLC isoforms, in fact, have been shown not to significantly affect ATPase activity in rat fibres (Bottinelli et al., 1994b), and very probably this is the case also for human fibres (Stienen, Bottinelli, Reggiani unpublished observation). Therefore, similarly to what observed for other parameters, there must be some other determinant of ATPase activity, but MHC isoform composition.

Tension cost, the ratio between ATPase activity and P_o/CSA, is a parameter that informs on the amount of ATP split to develop a given amount of force. As specific tension of type I is only slightly lower than that of IIA and IIX fibres, whereas ATPase activity is much lower in type I than in type IIA and IIX, tension cost is more than 3 times lower in type I than in type IIA and IIX fibres (Stienen et al., 1996) (Fig. 7B). It is therefore more "convenient" to use type I than type IIA and type IIX fibres for isometric contractions.

As expected on the basis of previous experiments on small mammals, ATPase activity of human fibres increases with temperature in the range 12–30°C with a Q10 of ~2.6 (Stienen et al., 1996). As in the same temperature range Q10 for force is lower (~2.0), the tension cost significantly increases with increasing temperature, i.e. the economy of force maintenance decreases when temperature rises. Therefore, the cost of force developing at physiological body temperature might be much higher than expected on the basis of determinations at 12°C (Stienen et al., 1996).

3.1.3.2. ATP consumption during shortening and thermodynamic efficiency. Since the classical experiments by Fenn (1923), it is known that during shortening the rate of energy liberation increases above isometric values, i.e. the "Fenn" effect occurs. The Fenn effect has been described in detail in whole muscles of the frog (Woledge, 1968; Irving and Woledge, 1981; Woledge et al., 1985) and more recently in single frog muscle fibres by determining the extra amount of heat released with a very sensitive thermopile (Woledge et al., 1988; Linari and Woledge, 1995). In skinned single fibres the thermopile cannot be utilized and the determination of the extra ATP consumed during shortening is technically difficult due to the short duration of shortening and to the low absolute values of extra energy consumed. However, such determinations have been recently done in single fibres of rabbit and rat using a spectrophotometric method (Potma and Stienen, 1996; Reggiani et al., 1997) and a fluorescent Pi

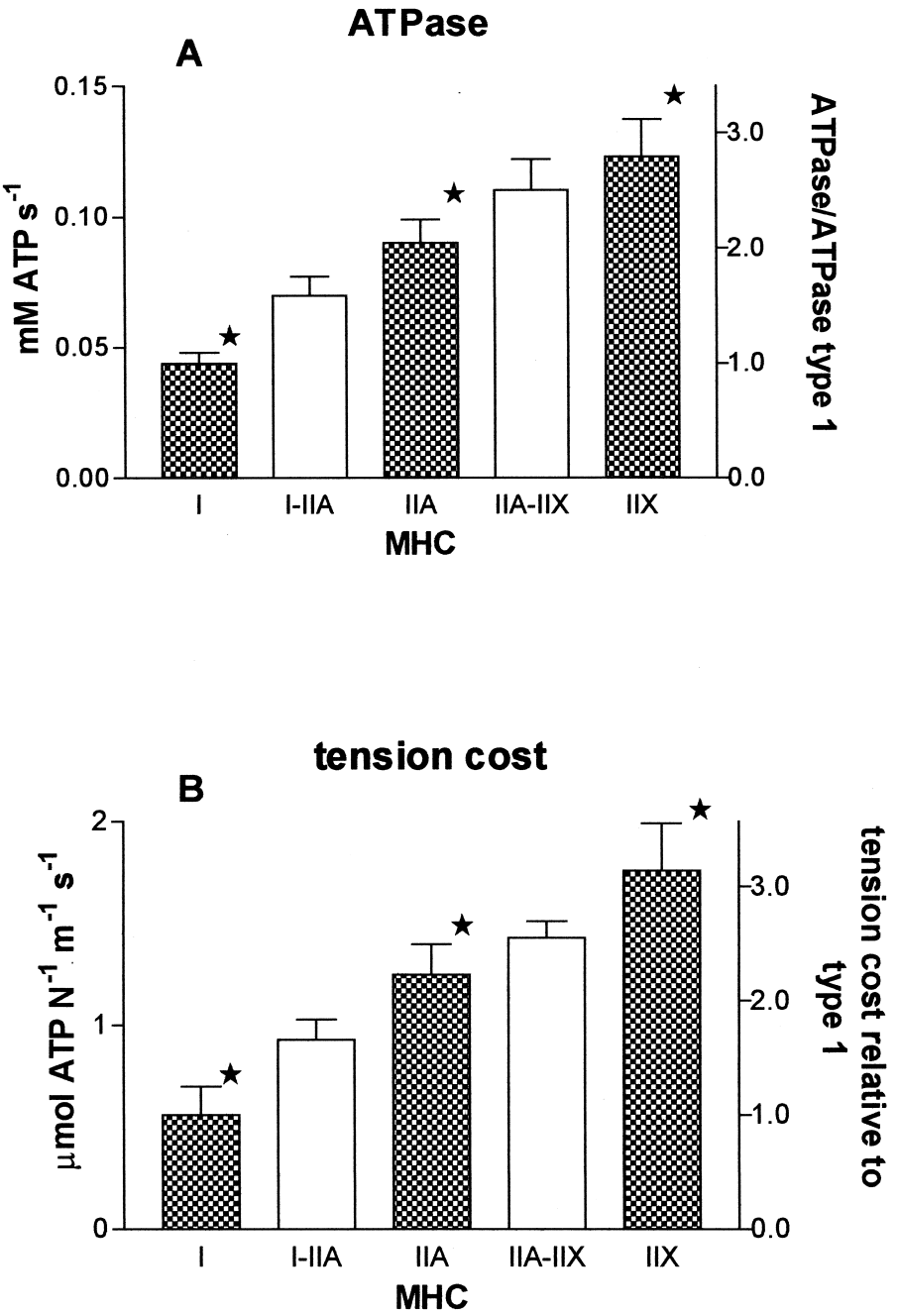

Fig. 7. ATPase activity in isometric conditions (panel A) and tension cost (panel B) of human skeletal muscle fibres. Fibres are classified on the basis of MHC isoforms composition determined by SDS-PAGE. ATPase is determined in skinned fibres using a spectrophotometric method. Tension cost is the ratio between ATPase and tension. Temperature, 12°C; sarcomere length, 2.5 μm. Stars indicate groups that are significantly different. Data from Stienen et al. (1996).

probe (He et al., 1999), and have confirmed that ATP consumption increases several times during shortening.

From the amount (or rate) of extra energy liberated from ATP hydrolysis, and from the amount of work (or power) produced, efficiency can be determined. Contrasting results have been obtained comparing efficiency of slow and fast muscles of small mammals. Efficiency in slow muscles has been found to be greater than in fast muscles (Gibbs and Gibson, 1972; Wendt and Gibbs, 1973), smaller (Heglund and Cavagna, 1987) or similar (Barclay et al., 1993). Two recent studies have analyzed the ATP consumption rate during shortening and the efficiency in single muscle fibres with different MHC isoform composition (Reggiani et al., 1997; He et al., 2000). In rat fibres using a spectrophotometric assay ATP consumption has been found to increase during shortening in all fibre types, the increase being proportional to shortening velocity and to the isometric ATPase rate (Reggiani et al., 1997). Type I fibres, therefore, not only had lower ATPase rate under isometric conditions (see above), but also had lower ATPase rate during shortening than IIX fibres. Thermodynamic efficiency (power/rate of free energy liberation by ATP) reached a maximum at a velocity similar to that at which maximum power was developed, above and below such velocity efficiency decreased. Interestingly, in the rat, thermodynamic peak efficiency of type I fibres (40%) was higher than that of type IIX fibres (30%). Thus, the diversity in peak efficiency was small (0.3-fold) in comparison to the much larger differences observed for W_{max} (7-fold) and for all other contractile properties, for isometric ATPase and especially tension cost.

In human fibres using a fluorescent Pi probe ATP consumption rate has been measured during isovelocity releases and efficiency has been calculated (He et al., 2000). The results are mostly consistent with studies on small mammals. Peak thermodynamic efficiency of slow fibres (21%) appears not statistically different from that of IIA and IIA/IIX (hybrid) fibres (26%) (Fig. 8; pure IIX fibres were not found; in Fig. 8 only type I and type IIA fibres are shown for the sake of clarity). Interestingly, therefore, both in rats and in humans, fibre types that differ very widely for most contractile properties, and that especially develop very different power (7-fold in rat, 10-fold in humans) at very different optimal velocity (7-fold), have similar thermodynamic efficiency. The picture emerging from skinned fibre studies in small mammals and humans strongly suggest that the diversity between myosin isoforms produces large variations in most contractile parameters (V_o, W_{max}, tension cost) and in ATPase activity, but very little variations in other parameters such as efficiency (and isometric force). This might suggest that all sarcomeric myosins have a common conserved basic mechanism of chemo-mechanical transduction on which the modulation of the kinetic properties is superimposed. Interestingly, increasing temperature from 12°C to 20°C, ATP consumption during shortening ($Q10 = 2.0–2.2$) increases less than power output ($Q10 = 5.3–5.4$) and therefore thermodynamic efficiency increases (from 21% to 33–35% in slow fibres and from 26% to 40% in fast fibres (He et al., 2000) (Fig. 8). This is surprising as, in isometric conditions, an increase in temperature decreases the economy of force production, i.e tension cost increases (Stienen et al., 1996a).

The difference in ATP consumption between slow and fast fibres, and the constancy of thermodynamic efficiency among different fibre types might have important consequences in vivo. It is expected, in fact, that the efficiency of chemo-mechanical energy transduction will be constant regardless of the speed of movement and the recruitment of fast or slow motor units/

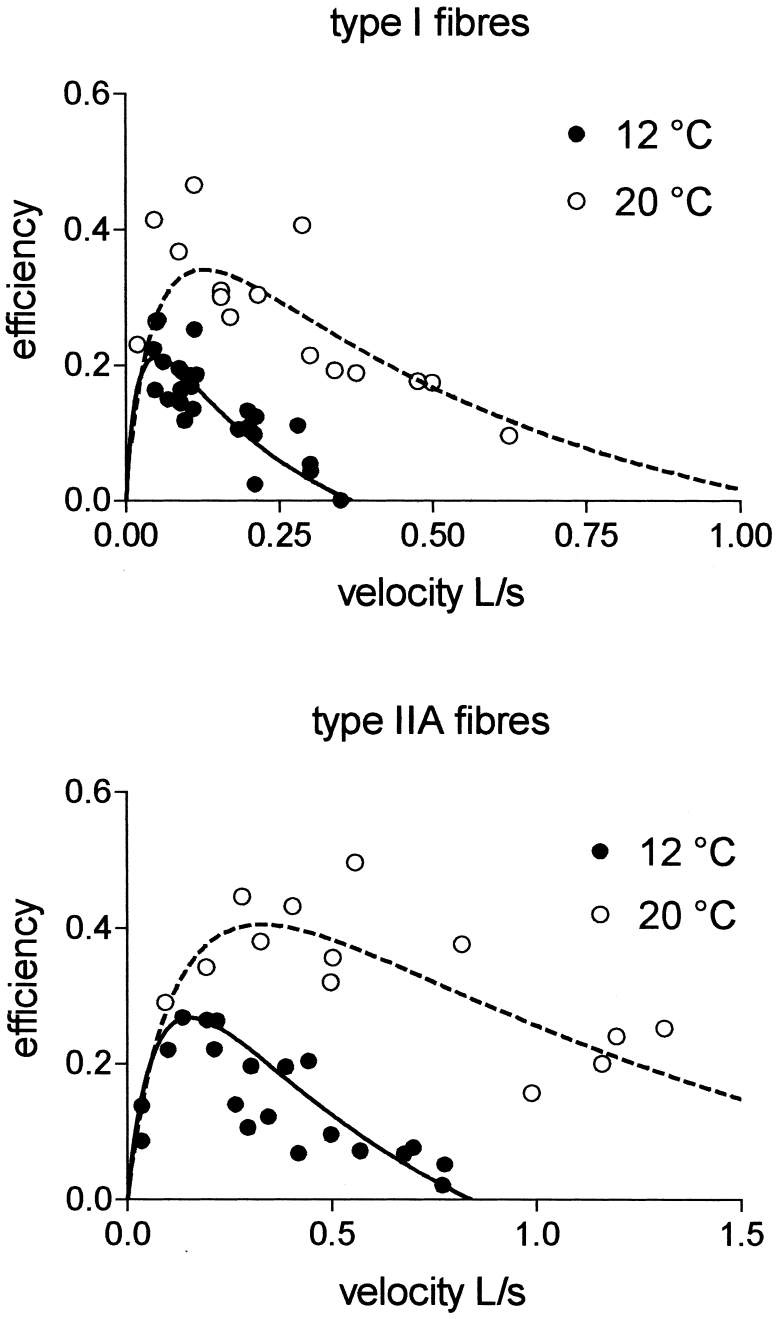

Fig. 8. Relation between thermodynamic efficiency of human skeletal muscle fibres at 12°C and 20°C and velocity of shortening. Upper panel: type I fibres; lower panel: type IIA fibres. Full circles 12°C, empty circles 20°C. Fibres are classified on the basis of MHC isoform composition determined by SDS-PAGE. Efficiency is calculated from the ratio between power and free energy change associated with ATP hydrolysis. Data from He et al. (2000).

fibre types, provided that shortening occurs at speeds close to optimal velocity. When fast fibre types are recruited, in fact, ATP consumption is higher, but power produced is also higher and efficiency can remain constant.

3.1.4. Calcium sensitivity and regulatory proteins

In human skeletal muscles, individual fibres develop force and relax at different rates and respond differently to a change in the rate of stimulation, i.e. have different twitch/tetanus ratio and force frequency relation (Eberstein and Goodgold, 1968; Buchthal and Schmalbruch, 1970). At cellular level main determinants of such fibre heterogeneity are: the sensitivity of myofibrillar apparatus to Ca^{++}, the rate at which tension is developed when actin and myosin interact in steady state conditions, the rate of Ca^{++} uptake and release by the sarcoplasmic reticulum. Possible differences in the rate of force developing have been considered above (A2 Isometric force and rate of tension rise). Ca^{++} release and uptake by sarcoplasmic reticulum will be dealt with below (Section 3.2.2). Here Ca^{++} sensitivity will be considered.

The sensitivity of myofibrillar apparatus to Ca^{++} is generally studied in skinned fibres determining the isometric force developed at steady state in activating solutions of different calcium concentrations, i.e. building force-pCa relationships. Force-pCa can be fitted by Hill's equation [relative tension $= (K[Ca^{++}])^n/(1+(K[Ca^{++}]))^n$] and two parameters that quantify calcium sensitivity can be calculated: pCa50% and Hill coefficient (n). The value of pCa50% (i.e. the pCa value at which 50% of maximum tension is developed) is an index of myofibrillar affinity for Ca^{++}, whereas n is an index of the steepness of the relationship and therefore of the level of cooperativity in the myofibrillar activation.

In small mammals, at room temperature, type I fibres have higher calcium sensitivity than IIX fibres, and IIX fibres higher calcium sensitivity than IIA fibres, and Hill coefficient is lower for type I than for type IIX and IIA fibres (Stephenson and Williams, 1981; Danieli-Betto et al., 1990). Measurements in human fibres are consistent with animal data as regards Hill's slope, i.e. type I fibres have lower n values than IIA and IIX fibres (Fink et al., 1990; Ruff and Whittlesey, 1991; Lynch et al., 1994; Widrick et al., 1996a; Bottinelli et al., 1998) (Fig. 9). It is, however, much less clear whether or not human fibres differ as regards calcium sensitivity. No significant differences in pCa50% have been found by Fink et al. (1990) (Fig. 9) and by Lynch et al. (1994) at 22°C, whereas Widrick et al. (1996a) and Ruff and Whittlesey (1991) reported higher calcium sensitivity for type I than for type IIA human fibres at 15°C and 22°C respectively. Finally, Bottinelli et al. (1998) reported higher calcium sensitivity for type IIA and IIA-IIX than for type I human fibres at 12°C. Such discrepancies might be accounted for, at least in part, by the different temperature at which calcium sensitivity was assessed. In fact, in rat fibres, whereas Hill coefficient is hardly affected by temperature changes (Stephenson and Williams, 1981), calcium sensitivity is significantly affected. With increasing temperature, the calcium sensitivity decreases much more in fast fibres than in slow fibres (Stephenson and Williams, 1981; Harrison and Bers, 1990). Should this be the case also in human fibres, the lower calcium sensitivity of type I fibres at 12°C and the higher calcium sensitivity of type I fibres at 15–20°C in comparison to fast fibres might be explained. The issue of whether or not human fibres have different calcium sensitivity is relevant for at least two general reasons: (1) as motor units in vivo are in most movements

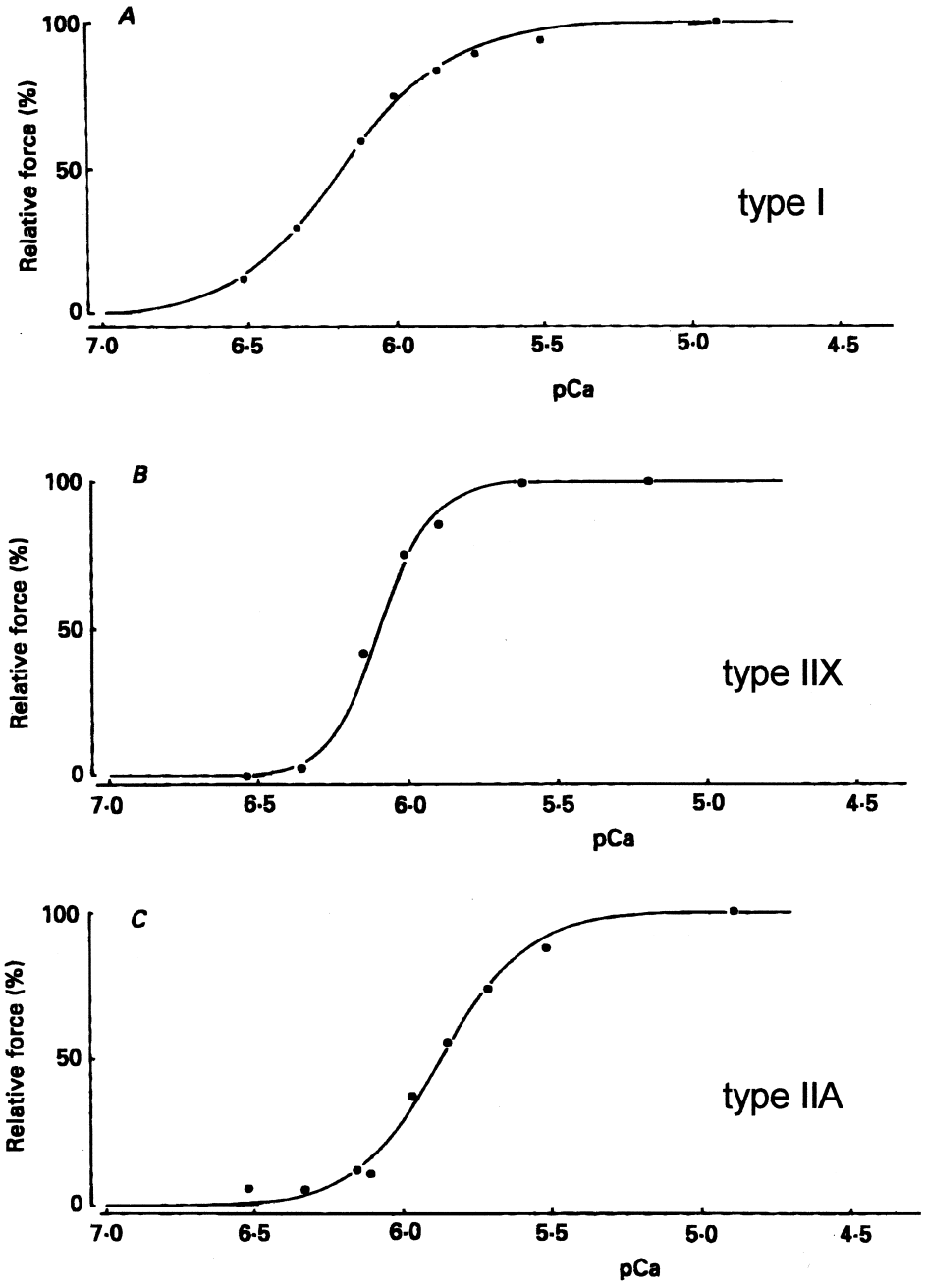

Fig. 9. Force–pCa curves of single human muscle fibres. Panel A: Type I fibre, Hill slope = 2.3. Panel B: type IIB fibre, Hill slope = 5. Panel C: type IIA fibres, Hill slope = 3. Calcium sensitivity of type I, IIA and IIX fibres was not significantly different. Fibres are classified on the basis of diversity in strontium sensitivity. Figure redrawn and modified from Fink et al. (1990).

submaximally activated (Belanger and McComas, 1981), different calcium sensitivity of type I, IIA and IIX fibres would contribute to determining differences in force development; (2) it can help in establishing which factors are involved in determining and modulating calcium sensitivity.

Although it is generally believed that Ca^{++} sensitivity is mainly determined by the regulatory proteins tropomyosin and troponin, other proteins are surely involved as well. As binding of myosin S1 fragment to regulated actin facilitates further binding and stabilizes the thin filament in a state of higher calcium sensitivity (Greene and Eisenberg, 1980; Guth and Potter, 1987), it cannot be ruled out that different MHC isoforms differentially affect calcium sensitivity. Extraction of myosin binding protein C (MBP-C) decreases activation at submaximal Ca^{++} concentrations (Hofmann et al., 1991) suggesting a possible role for MBP-C in determining Ca^{++} sensitivity. A clear picture of how Ca^{++} sensitivity is modulated is still missing not only in human fibres, but even in skeletal fibres of small mammals that have been studied in much more detail. This is not surprising given the complexity of the tropomyosin–troponin isoform system that is probably the main myofibrillar protein system controlling calcium sensitivity. Three TM isoforms, two TnI isoforms, two TnC isoforms and several TnT are expressed in skeletal muscles. Notwithstanding a coordinated expression of TM and Tn isoforms has been suggested (Schachat et al., 1985; Danieli-Betto et al., 1990; Bottinelli et al., 1998), the issue is still unclear and requires further attention. Some information on the role of TM and Tn isoforms in modulating Ca^{++} sensitivity is available in rabbit (Schachat et al., 1987) and also in humans (Bottinelli et al., 1998). Schachat et al. (1987) suggest that the higher the relative proportion of Tnt-2f and TM-α-fast the higher is calcium sensitivity in rabbit fibres. However, fibres were not identified as regards MHC isoform composition and differences in Ca^{++} sensitivity could be due to differences in myosin isoforms. Bottinelli et al. (1998) report the first identification of both MHC and TM isoform composition in the same human muscle fibres. However, as the aim of their work was to study the possible impact of a TM mutation on Ca^{++} sensitivity, only IIA fibres were analyzed in detail, and little was concluded on the role of TM isoforms in determining Ca^{++} sensitivity.

Lowering pH decreases calcium sensitivity (decreases pCa50%), increase the threshold for contraction by Ca^{++} and increase cooperativity (Hill coefficient) in human muscle fibres as previously shown in rat and rabbit (Lynch et al., 1994). More recent studies, however, have cast doubts whether these effects of acidosis are present at physiological temperatures (Pate et al., 1995; Westerblad et al., 1997). No relevant changes in Ca^{++} sensitivity and cooperativity of human muscle fibres are observed following sprint training (Lynch et al., 1994).

Interestingly, studies on point mutation of TM and Tn linked to Familial Hypertrophic Cardiomyopathy may provide some clue on the role of such proteins in determining calcium sensitivity. That a point mutation (Asp175Asn) in α-fast TM is associated to an increase in Ca^{++} sensitivity (Bottinelli et al., 1998) supports the idea that TM is a major determinant of calcium sensitivity.

A more detailed analysis is required to reach a conclusion on the differences in calcium sensitivity among different human fibre types especially at or near body temperature. As with small mammals, the analysis of myosin, TM and troponin isoform composition of human fibres of known pCa50% and Hill coefficient will be necessary to clarify how calcium sensitivity is modulated.

3.1.5. Passive properties and length–tension relation

Mammalian skeletal muscle fibres are slack at sarcomere lengths (SL) 2.0–2.5 μm. Above this length they can be elongated by ~0.5 μm with little increase in tension. Further elongations cause a sharp increase in tension up to a plateau between 3.8–4.8 μm (yield length) (Wang et al., 1991). It is now generally accepted that titin is the main determinant of resistance to elongation. This was first demonstrated by the reduction in passive tension following removal of titin (Horowits et al., 1986; Higuchi, 1992; Granzier and Irving, 1995). Recent results of molecular mechanics have shown that stress–strain properties of single titin molecule are comparable with those of muscle fibres (Kellermayer et al., 1997; Trombitas et al., 1998). Diversity in passive tension–length curve of isolated myofibrils have been demonstrated in rabbit muscle (Linke et al., 1996) and this has been related to diversity of titin isoforms (Wang et al., 1991; Linke et al., 1996). As discussed above, titin is encoded by a single gene but can give origin to multiple isoforms by alternative splicing. Relevant to passive elastic properties is the length of the repetitive tandem IgG region, the length of the PEVK element and the presence of the two elastic sites N. Cardiac muscle titin exhibits the shortest repetitive region and the shortest PEVK region: this determine a low slack length and a steep increase in resting tension upon elongation (Granzier and Irving, 1995; Trombitas et al., 1995). Both repetitive domain and PVEK region are shorter in fibres from fast muscles (psoas, gastrocnemius) than in slow muscle (soleus) (Labeit and Kolmerer, 1995b). This accounts for the observed higher resting tension at the same SL of fast versus slow rabbit fibres (Horowits, 1992; Linke et al., 1996).

Little is known about passive properties of human skeletal muscle fibres. Most single fibre experiments have been performed at slack length that corresponds to ~2.5 μm SL. Above such length, 2.55–2.75 μm, some resting tension appears (Larsson and Moss, 1993). No differences in slack length have been reported so far between type I, IIA and IIX fibres (Hilber and Galler, 1997). Similar resting tensions have been observed for slow and fast fibres by Horowits et al. (1990) [see also Horowits (1992)]. It is on the other hand demonstrated that slow fibres in human soleus express a "long" titin isoform as the rabbit soleus fibres (Labeit et al., 1997).

3.2. Excitability and E–C coupling

3.2.1. Diversity in electrical membrane properties and ion channels

Human muscle fibres differ in their excitability and in their ability to follow the rate of motor neuron discharge. The presence of specific ionic channels and transport systems in the muscle fibre membrane or of different proportions of the same systems can be responsible for this diversity.

The functional demands placed by the motor neuron discharge rates on membrane properties of muscle fibres are very different. Slow fibres must be able to generate action potentials during prolonged firing at low rate, without loosing excitability in spite of the accumulation of potassium in the extracellular space and particularly in the T tubules. Fast fibres, and among them IIX fibres more than IIA fibres, need to recover excitability quickly after each action potential, but do not need to maintain excitability for long periods as fast motor unit discharge occurs in short bursts of high frequency (Henneman et al., 1974; Monster et al., 1978; Bawa et al., 1984).

In human muscles the resting potential does not show significant differences between IIX (−94.6 mV) and IIA fast muscle fibres (−92.7 mV) (Ruff and Whittlesey, 1993), but is less negative in slow fibres (−85.3 mV) (Ruff and Whittlesey, 1992; Ruff, 1996). The membrane capacitance seems to be similar in fast and slow fibres (3.82 vs 3.76 microF/cm^2) (Ruff and Whittlesey, 1992). The membrane resistance per unit area is also not different between fast and slow fibres.

At variance of neurons where membrane ionic conductance is dominated by potassium, 70% of the conductance of muscle fibre membrane is due to chloride ions (Bretag, 1987) and only 30% to potassium. At least two isoforms of *chloride channels* are expressed in muscles: the voltage gated ClC1 which is muscle specific and the ubiquitous ClC2 (Table 4) (Pusch and Jentsch (1994)). The voltage gated ClC1 has an important role in repolarizing the fibres after an action potential: mutations of the gene coding for this channels cause various forms of myotonia (Cannon, 1996). No evidence of heterogeneous expression in different fibre types is available.

Na channels play a central role in determining excitability of muscle fibres. Their role is also confirmed by the dramatic alterations which follow point mutations of sodium channels as in paramyotonia and periodic paralysis [see for recent reviews (Cannon, 1996; Lehman-Horn and Rudel, 1997)]. Excitability of the membrane is determined by the number of Na channels and by the fraction of them which is not inactivated. The density of Na channels is higher in fast than in slow fibres in humans (Ruff and Whittlesey, 1992) as in other mammals (Caldwell et al., 1986). This is consistently supported by evidence obtained in electrophysiological studies as well as in saxitonin binding experiments (Hansen-Bay and Strichartz, 1980). In each fibre, Na current density is higher at the border of the endplate than away from it: 4–7 times in IIX fibres, 3–7 times in IIA fibres (Ruff and Whittlesey, 1993). This should ensure that an action potential is triggered every time acetylcholine release produces an end plate potential. The decrease in Na current moving away from the endplate is much lower in slow fibres (Ruff and Whittlesey, 1992). No local differences (close and away from the endplate) have been found in the voltage dependence of the activation and inactivation processes (Ruff, 1996).

Na channels in human fibres show fast and slow inactivation processes. In fast fibres slow inactivation reduces Na current to about 50% at resting membrane potential. Its time constants is in the order of 20–30 s (Ruff and Whittlesey, 1992, 1993). Fast inactivation acts much faster (time constants in the order of 0.1–1 ms) and at less negative potentials. Comparing fast and slow fibres, fast inactivation acts at potential less negative in slow ($Vh1/2 > -65$ mV) than in fast fibres ($Vh1/2 < -66$ mV) and the same is true for slow inactivation ($Vs1/2 = -71.7$ mV in slow and $= -93.1$ mV in fast fibres) (Ruff and Whittlesey, 1992). The time course of slow inactivation might render a muscle fibre unexcitable for few minutes. The more negative value of Vs1/2, in particular, can make fast fibres unexcitable in the presence a modest depolarization which could follow an increase in extracellular potassium concentration during repetitive activations (Ruff, 1996). This is avoided in slow fibres as Vs1/2 is less close to the resting potential.

Only one gene (George et al., 1992) codes for the Na Channel alfa subunit expressed in human muscle fibres (Table 4). The origin of the diversity between fast and slow fibres might be searched in beta subunits, in the anchoring system to sarcolemma or in post-transcriptional

Table 4
Proteins of human skeletal muscle involved with excitability and excitation–contraction coupling and their genes in human genome

Protein	Expression	Locus	Gene	Reference
Chloride channel 1	skeletal muscle	7q32-qter	ClCN1	Koch et al. (1992)
Chloride channel 2	skeletal muscle and other tissues	3q26-qter	ClCN2	Cid et al. (1995)
Na channel α-subunit	skeletal muscle	17q23-q25	SCN4A	George et al. (1993)
Na channel β-subunit (β1)	skeletal muscle	19q13	SCN1B	Isom et al. (1992)
K channel kH1 (voltage gated)	skeletal muscle	2p25	KCNF1	Su et al. (1997)
K channel kH2 (voltage gated)	skeletal muscle	20q13	KCNG1	Su et al. (1997)
K channel Kir4.2 (inward rectifier)	skeletal muscle	21q22.2	KCNJI5	Gosset et al. (1997)
K channel Kir6.2 (inward rectifier)	skeletal muscle	21q22.1	KCNJI6	Sakura et al. (1995)
SUR2A (together with Kir6.2KATP)	skeletal muscle	12p12.1	SUR2	Chutkow et al. (1996)
Na–K ATPase alpha1	skeletal muscle	1p13	ATP1A1	Ruiz et al. (1995)
Na–K ATPase alpha2	skeletal muscle	1q21-q23	ATP1A2	Yang-Feng et al. (1988)
Na–K ATPase alpha3	skeletal muscle	19q13.2	ATP1A3	Ovchinnikov et al. (1988)
Na–K ATPase beta1	skeletal muscle	1q22-q25	ATP1B1	Ruiz et al. (1995)
Na–K ATPase beta2	skeletal muscle	17p	ATP1B2	Martin-Vasallo et al. (1989)
DHPR α-1 S-subunit	skeletal muscle	1q32	CACNA1 S	Gregg et al. (1993)
DHPR β-subunit	skeletal muscle	17q21-q22	CACNLB1	Iles et al. (1993)
DHPR γ-subunit	skeletal muscle	17q24	CACNLG	Iles et al. (1993)
Ryanodine receptor type I	skeletal muscle	19q13.1	RYR1	Trask et al. (1993)
Ryanodine receptor type 2	cardiac muscle	1q42.1-43	RYR2	Tunwell et al. (1996)
Ryanodine receptor type 3	nervous system and skeletal muscle	15q14-q15	RYR3	Leeb and Brenig (1998)
Calsequestrin 1	fast skeletal muscle fibres	1q21	CASQ1	Otsu et al. (1993)
Calsequestrin2	cardiac muscle	1p13.3-p11	CASQ2	Otsu et al. (1993)
Parvalbumin	fast fibres	22q12-q13.1	PVALB	Fohr et al. (1993)
SERCA1	fast fibres	16p12.1	ATP2A1	Zhang et al. (1995)
SERCA2	slow fibres, cardiac muscle	12q23-q24.1	ATP2A2	Sakuntabhai et al. (1999)
Phospholamban	slow fibres, cardiac muscle	6q22.1	PLN	Otsu et al. (1993)

(alternative splicing) or post-translational (e.g. phosphorylation) modification of the alfa subunit (Ruff and Whittlesey, 1993).

K currents in human fibres are rather small (Almers et al., 1984): this might be relevant to minimize the increase of extracellular potassium following repetitive stimulation. Due to its small amplitude the contribution of potassium current to repolarization is probably less important than the contribution of sodium channel inactivation. Inward rectifying, voltage gated, Ca-activated and ATP-sensitive K channels are present in muscle fibres. The ATP-sensitive K channel is probably the most abundant K channel active in the skeletal muscle fibers of humans and animals and is a tetrameric product of an inward rectifying channel (Kir6.2) and a sulfonylurea receptor (SUR2A) (Table 4) (Aguilar-Bryan et al., 1998). A diversity in potassium delayed outward current has been described between fast and slow fibres of the rat: two components (one slow and one fast) are detectable in slow fibres, whereas only the fast component is present in fast fibres (Duval and Leoty, 1980). No evidence of diversity among fibre types is available for human muscle fibres.

Relevant to the ability to control sodium and potassium concentrations during repetitive firing is the activity of sodium-potassium pump. The *Na–K pump* is a tetramer, composed by two α subunits and two β subunits (Table 4). Three isoforms of the α subunit (α1, α2, α3) and two (β1, β2) of the three isoforms of the β subunit are expressed in mammalian muscles. In the rat type I and type IIA fibres express α1 and α2 together with β1 (dimers: α1-β1 and α2-β1), whereas type IIX fibres express α1 and α2 together with β2 (dimers α1-β2 and α2-β2) (Hundal et al., 1993). In human muscles slow fibres express α1, α2 and α3 associated with β1 (Hundal et al., 1994).

The density of the Na–K pump measured from oubain binding indicates that, at least in rat muscles, fast fibres contain about 20% more pumps than slow fibres (Everts and Clausen, 1992). This would compensate the Na influx during action potentials which is expected to be greater in fast than in slow fibres in relation to the greater density of Na channels (see above). However, the increase of intracellular Na concentration which accompanies electrical muscle stimulation enhances pump activity three times more in slow than in fast fibres: the activity-dependent increase in intracellular sodium concentration is thus higher in slow than in fast muscles (Everts and Clausen, 1992).

The capacity for potassium uptake in human muscles is estimated to be 70 micromol/kg ww/s (Norgaard et al., 1984). No information is available about Na–K pump density in single human fibres. It has been reported that training causes an increase of the concentration or of the activity of Na–K pump in human muscles [reviewed by McKenna et al. (1996)].

3.2.2. Diversity in the systems devoted to Ca-regulation

A difference between slow and fast fibres in the calcium transient following a single stimulus has been demonstrated in rat skeletal muscles (Eusebi et al., 1980; Fryer and Neering, 1989). Calcium transient in fast fibres reaches a higher peak and declines faster than in slow fibres. Although similar evidence is not available for human muscle fibres, voltage clamp experiments on cut fibres show a larger and faster calcium transient in fast than in slow fibres (Delbono et al., 1995).

In relation to the smaller size of the calcium transient in slow fibres, the twitch/tetanus ratio is also lower in slow than in fast twitch fibres (Close, 1972; Eberstein and Goodgold, 1968;

Buchthal and Schmalbruch, 1970) in human muscles; and seems a likely explanation in the smaller size of the calcium transient in slow fibres. As discussed above (Section 3.1.4) the relation between calcium transient amplitude and twitch amplitude must take into account the higher calcium sensitivity of slow fibres at the physiological or quasi-physiological temperatures (Stephenson and Williams, 1982). The longer time to peak tension and the longer duration of the relaxation in slow compared to fast fibres [(Eberstein and Goodgold, 1968; Buchthal and Schmalbruch, 1970) for humans] corresponds to the slower time course of the intracellular calcium transient and to slower cross bridge kinetics of the slow myosin isoform.

The molecular basis of the difference in calcium transients can be traced to the diversity of the molecules involved in calcium release (Table 4). *DHPR*, i.e. the calcium channel which acts as a voltage sensor during excitation contraction coupling is a complex of 5 subunits: $\alpha 1$, $\alpha 2$, β, γ, δ. The skeletal muscle isoform of the $\alpha 1$ subunit is expressed in all skeletal muscle fibres and is essential for the direct activation of calcium release through the ryanodine receptor. A light (190 kD) and a heavy (212 kD) form of $\alpha 1$ skeletal muscle subunit have been identified (De Jongh et al., 1991), but nothing is known about their distribution in individual fibres. The density of DHPR per volume unit is 3–5-fold greater in fast twitch than in slow twitch fibres [(Delbono and Meissner, 1996) rat; (Renganathan et al., 1998) mouse] and in accordance with this charge movement is much greater in fast than in slow fibres (Dulhunty and Gage, 1983). A decline of the ratio DHPR/RyR has been observed in human muscle fibres during aging (Delbono et al., 1995).

Two isoforms of the sarcoplasmic calcium release channel/ ryanodine receptors (*RyR*) are expressed in mammalian skeletal muscle fibres: RyR1 is the dominant one, whereas expression of RyR3, after a wide distribution during the postnatal period, is restricted to few muscles (Sorrentino and Reggiani, 1999). There is also evidence of a differential distribution of RyR3 in individual muscle fibres (Flucher and Sorrentino, 1999), but it is not yet clear whether expression of RyR3 is related to a specific fibre type. In human muscles ryanodine receptors have received considerable attention as mutations of RyR1 are responsible for malignant hyperthermia [MH, see for a review (Mickelson and Louis, 1996)]. Nothing, however, is known about RyR isoform expression in human muscles.

Differences in the kinetics of RyR have been found comparing RyR isolated from fast and slow muscles and incorporated into lipid bilayers (Lee et al., 1991), but it is not clear whether this is related to specific isoforms or to post-translational modifications (e.g. phosphorylation). Also not clear is whether the ratio between DHPR and RyR is lower in slow than in fast fibres (Lamb, 1992; Margreth et al., 1993). This would imply that the proportion of RyR directly controlled by DHPR through the charge movement is lower in slow than in fast fibres (Delbono and Meissner, 1996). In this case calcium-induced calcium release would play a more important role in slow than in fast fibres and excitation–contraction coupling in slow fibres might be therefore reminiscent of that typical of cardiac muscle.

Ca-release from SR in slow fibres is less inhibited by cytosolic Mg (Stephenson et al., 1998): this might contribute to a lower sensitivity to fatigue as an increase of intracellular Mg concentrations occur during advanced stages of fatigue. The explanation might be found in different RyR isoforms, in different RyR density or in a greater concentrations of calcium inside the SR of slow fibres. This would also explain the greater effect of caffeine in slow fibres, as suggested by Stephenson et al. (1998). Actually, a greater sensitivity to caffeine of slow

compared to fast fibres has been observed in rat fibres (Fryer and Neering, 1989) and confirmed in human fibres (Adnet et al., 1993). The threshold for caffeine contracture is often used as a test of malignant hyperthermia (MH) diagnosis: in normal human muscles the threshold is in type I fibers 5.89 ± 1.8 mM in type II fibers is 10.46 ± 2.6 mM (Adnet et al., 1993) and is considerably decreased (below to 2 mM) in muscles from MH subjects (EMHG, 1984).

In view of the different capacity to store calcium, it is interesting that in the sarcoplasmic reticulum of slow fibres two isoforms of calsequestrin can be found (calsequestrin1 and calsequetrin2), whereas only one (calsequestrin1) is present in fast fibres (Table 4) (Damiani and Margreth, 1994; Yano and Zarain-Herzberg, 1994).

The faster decline of calcium transient in fast fibres can be due to a combined action of cytoplasmic buffers and calcium transport mechanisms. *Parvalbumin* is a cytosolic calcium buffer. In mammalian muscles parvalbumin is expressed in fast fibres at concentrations of about 1 mM, whereas it is virtually absent in slow fibres (Gundersen et al., 1988). Recent expression studies show that in human muscles parvalbumin expression is restricted to intrafusal fibres (Table 4) (Fohr et al., 1993). Troponin C is present in the myofibrils with two different isoforms: TnC-fast expressed in fast fibres has four calcium-binding sites (two high affinity and two low affinity), whereas TnC-cardiac-slow expressed in slow fibres lacks one of the low affinity sites (see above). Thus, the cytoplasmic buffer capacity for calcium is greater in fast compared to slow fibres.

The active *calcium transport* mechanism into the sarcoplasmic reticulum is also based on two isoforms one of them expressed in fast fibres (SERCA1) and the other expressed in slow fibres (SERCA2) (Table 4) (Lytton et al., 1992). The latter is regulated by the phosphorylation state of the regulatory subunit phospholamban (Hawkins et al., 1994). The density of the pump is much greater (5–7-fold) in fast than in slow fibres [(Leberer and Pette, 1986), rabbit; (Everts et al., 1989), human; (Wu and Lytton, 1993), rat]. This ensures a faster and more efficient removal of calcium in fast than in slow fibres. A difference of about 2 times in the rate of calcium uptake by SR has been found by Salviati et al. (1982b, 1984) between fast and slow human muscle fibres. No significant difference has been observed between IIA and IIX fibres.

3.3. Diversity in the energy production systems

The energy required by contractile activity is provided by ATP hydrolysis to ADP and Pi. ADP is then re-synthesized to ATP from PCr through the creatine kinase reaction, from glycolytic processes in the sarcoplasma and from oxidative phosphorylation in the mitochondria. Glycolytic processes represent the initial stages of glycogen and glucose metabolism and lead to pyruvate or lactate production. Pyruvate, fatty acid, ketone bodies provide supply of acetyl-CoA which is the substrate for the mitochondrial oxidative processes.

The histochemical determination of the metabolic enzyme activity in muscle fibres was initially developed by Ogata and Mori (1964) and by Padykula and Gauthier (1967). Different types of fibres were identified on the basis of the activity of the mitochondrial enzymes (SDH, COX, NADH dehydrogenase). In the muscles of small mammals three main types of fibres were classified: S or slow-twitch oxidative and FOG or fast twitch oxidative-glycolytic and FG or fast-twitch glycolytic (Barnard et al., 1971; Peter et al., 1972). The correlation between

motor unit studies with histochemical determination of enzymatic activity showed that: (1) there is a substantial homogeneity of metabolic properties among the fibres belonging to the same motor unit; and (2) enzymatic properties of the fibres can be correlated with resistance to fatigue: SO represent slow fatigue-resistant motor units, FOG and FG represent fast fatigue-resistant and fast fatiguable motor units, respectively (Burke et al., 1971, 1973; Kugelberg, 1973; Nemeth et al., 1981). More recent studies, however, gave evidence that even inside a motor unit variability in staining may occur and, at least in some cases, gradients related to the position of the fibres in the muscle can be observed (Kugelberg and Lindgren, 1979; Larsson, 1992). On the other hand variability of enzymatic properties along the length of individual fibres seems to be small (Pette et al., 1981).

The micro-determinations of enzymatic activities of single fibres were first developed by Lowry and by Essen and allowed to quantitatively assay various enzymatic activities in human muscle fibres (Essen et al., 1975; Lowry et al., 1978; Reichmann and Pette, 1982; Essen-Gustavsson and Henriksson, 1984). The distribution of the enzymatic activities determined in single fibres shows broad ranges of variations, where some peaks corresponding to groups of fibres with comparable activities are detectable (Reichmann and Pette, 1982) (Fig. 1). Determination of more than one enzymatic activity in the same fibres allows to observe direct correlations between enzymatic activities of the same pathways and inverse correlations between glycolytic and aerobic-oxidative enzymatic activities [see for example Fig. 10 (Rosser and Hochamba, 1993)].

More recently other micro-chemical methods were applied to human single fibres, producing a more complete picture of the inter-fibre diversity.

The basis of the diversity is in the first place structural: the oxidative aerobic metabolism finds its support in the oxygen supply from the capillary vessels, in the oxygen storage in myoglobin and in the oxygen utilization by the respiratory coenzyme chain. A diversity in capillary density has actually been demonstrated between aerobic oxidative and glycolytic fibres. In human muscles, the number of capillaries making contact with each fibre is higher for slow and IIA fibres (4.9 ± 0.3 and 4.5 ± 0.3) than for fast IIX fibres (3.5 ± 0.4) (Andersen, 1975). On the other hand the diversity in myoglobin content of human skeletal muscle fibres is small (Jansson et al., 1982; Svedenhag et al., 1983; Nemeth and Lowry, 1984). Myoglobin concentration in human muscles is 0.7 g/kg dw with a oxygen storage capacity of 0.4 mM (Nemeth and Lowry, 1984). A difference in mitochondrial content between fibre types and correlations between mitochondrial content and oxygen uptake and oxidative enzyme activities have been reported by several studies (Howald et al., 1985; Ingjer, 1979; Kiessling et al., 1974). The percentage of fibre volume occupied by mitochondria varies from 6% in type I fibres to 4.5% in type IIA fibres and 2.3% in type IIX fibres (Howald et al., 1985). Variations in mitochondrial content imply differences in the proportions of enzymes present in each fibre type. Furthermore, distinct mitochondrial shape and configuration for each human skeletal muscle fiber type were observed by Ogata and Yamasaki (1997).

A second source of diversity is the presence of specific isoforms of metabolic enzymes with different activities. For example five isoforms of lactate dehydrogenase (LDH) are expressed in rabbit muscles: slow fibres express all isoforms with a marked predominance of LDH-1, LDH-2, LDH-3, whereas in fast fibres LDH-5 is the predominant or in some case the only isoform expressed (Leberer and Pette, 1984). In human muscles an example of diversity based on

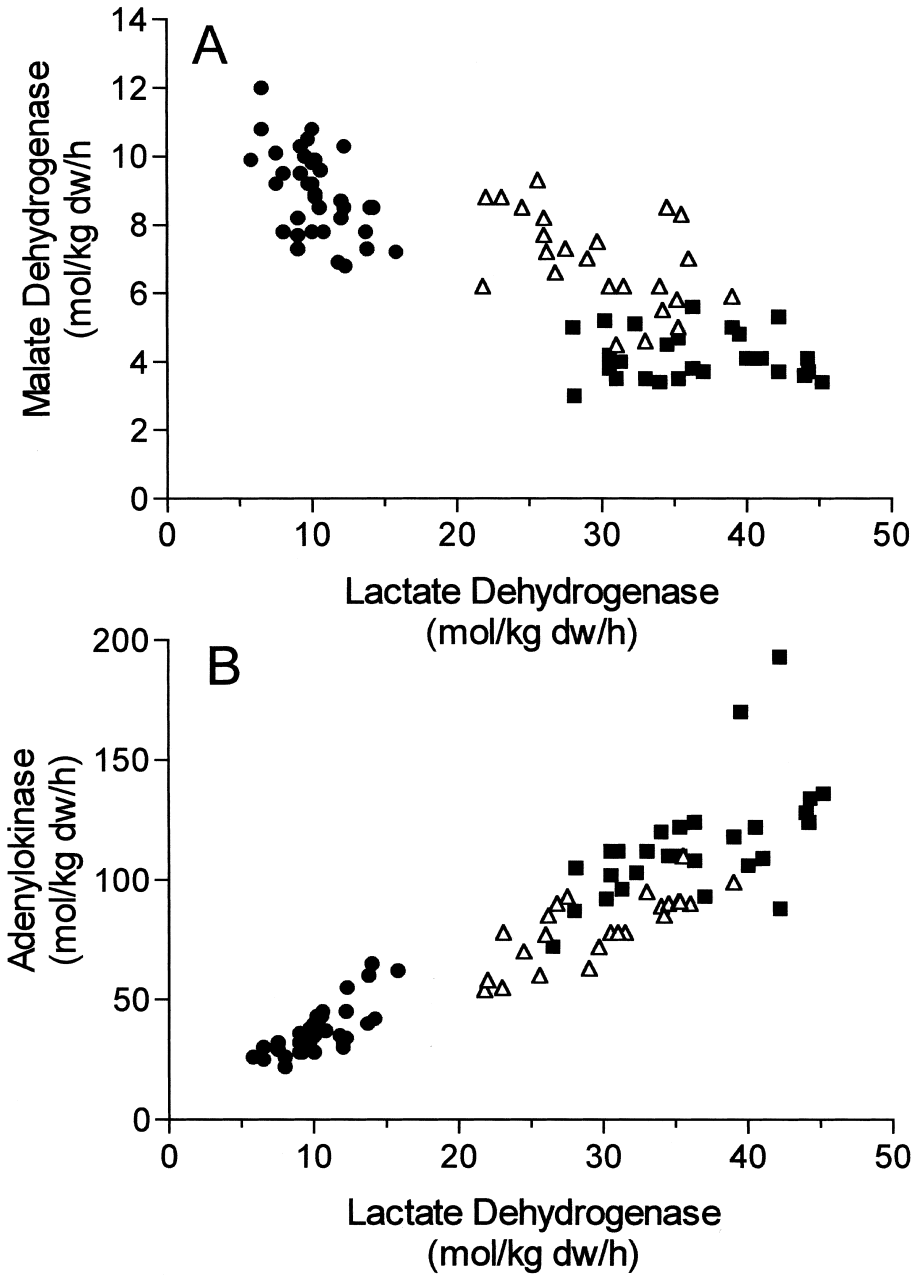

Fig. 10. Enzymatic activities of malate dehydrogenase (enzyme of the citric acid cycle), lactate dehydrogenase (enzyme which reduces pyruvate to lactate) and adenylokinase (enzyme which produces one ATP molecule from 2 ADP molecule, also called myokinase) determined in human single fibres. In each individual fibre two activities were determined and each fibre was classified on the basis of myofibrillar ATPase reaction as slow or type I (circles), fast IIA (empty triangles) and fast IIX (squares). Note the inverse relation between the activities of malate dehydrogenase and lactate dehydrogenase and the direct relation between adenylokinase and lactate dehydrogenase. Modified from Rosser and Hochamba (1993).

isoform distribution is represented by AMP deaminase (AMPD), an enzyme which produces IMP from AMP and whose activity is higher in glycolytic fibres (see below): several isoforms with a fibre-type specific pattern of expression have been reported (Morisaki and Holmes, 1993). A differential isoform distribution has been recently reported for enolase (2 phospho glycerate hydrolyase): the highly active β isoform is only expressed in fast fibres, whereas slow fibres contain the α isoform (Merkulova et al., 1999).

The presently available evidence indicates that the metabolic diversity involves many different aspects of muscle fibre metabolism from substrate availability to the rate of enzymatic processes of energy production and utilization. In the first place muscle fibres exhibit a different *availability of energetic substrates*. All muscle fibres contain stores of glycogen and lipids to be used as substrate for energy production, in addition to glucose, fatty acids and amino-acids which can be taken up from the blood supply. Glycogen content at rest is higher in fast than in slow fibres: determination based on optical density measurements on PAS stained section indicate that fast fibres contain 16% more glycogen than slow fibres (Vollestad et al., 1984). More recent microchemical determinations (Greenhaff et al., 1993) confirm that the content is greater (+31%) in fast (480 mmol/kg dw) than in slow fibres (364 mmol/kg dw). During exercise glycogen concentration decreases first in slow and thereafter in fast fibres: this probably reflects the orderly recruitment of motor units (Vollestad et al., 1984). On the other hand lipid content is greater in slow than in fast fibres. Slow fibres contain more triglycerides (7 mM) than fast fibres (4.2 mM) (Gollnick et al., 1981). Lipid droplets fill 0.5% of fibre volume in slow fibre and less than 0.1% in fast IIX fibres (Howald et al., 1985). Glucose supply also varies from fibre to fibre as glucose transport across the plasma membrane is different in slow and fast fibres.

Muscle fibres also differ in the availability of high energy phosphates. Several studies consistently indicate that in human muscles resting *PCr* content is higher in fast than in slow fibres. The reported values are range from 72 and 83 mmol/kg dw in slow and fast fibres respectively (Soderlund and Hultman, 1991) to 66 (slow fibres), 72 (IIA fibres), 90 (IIA-IIX fibres) mmol/kg dw (Sant'ana Pereira et al., 1996) and to 71.6 (slow fibres) and 86.4 (fast fibres) mmol/kg dw (Sahlin et al., 1997). Upon exercise the PCr content decreases to similar values both in fast and slow fibres (Soderlund and Hultman, 1991; Sahlin et al., 1997).

By contrast, resting *ATP* content is rather similar in slow and fast fibres: 25 mmol/kg dw (Soderlund and Hultman, 1991), 25.5 mmol/kg dw (Greenhaff et al., 1993) and 22–27 mmol/kg dw (Sant'ana Pereira et al., 1996). The ATP concentration is maintained relatively constant in both fibre types also during exercise (Sahlin et al., 1997). Only after contractile activity under extreme conditions (for example maximal electrical stimulation with occluded circulation) has a significant decrease been observed (Soderlund and Hultman, 1991).

ADP concentration in human muscle fibres has been estimated around 2–3 mmol/kg dw (Soderlund and Hultman, 1991; Sahlin et al., 1997). ADP concentration is determined by the creatine kinase equilibrium and is proportional to Pi/PCr ratio (Chance et al., 1985). ADP concentration can increase up to 4 mmol/kg dw after heavy exercise due to imbalance between ATP hydrolysis and ATP re-synthesis (Soderlund and Hultman, 1991). Free ADP concentration can increase even more: from 18–20 μM at rest to 140–180 μM after heavy exercise (Sahlin et al., 1997). No clear information is available on possible diversity among fibres.

Concentrations of *AMP and IMP* (which derives from AMP by deamination) are very low at rest [0.05–0.1 mmol/kg dw (Soderlund and Hultman, 1991)]. During intense exercise IMP concentration increases up to 3–10 mmol/kg dw (Soderlund and Hultman, 1991; Sahlin et al., 1997). The increase is inversely related to glycogen concentration (Sahlin et al., 1997) and might represent a signal to activate glycogenolysis [(Aragon et al., 1980), see also (Greenhaff et al., 1993; Sahlin et al., 1997) for human muscles].

Pi content is higher in slow than in fast fibres. In small mammals Pi concentration ranges between 0–2 mM in fast muscles and 3.6–6.7 in slow muscles (Kushmerick, 1992). In human muscles at rest Pi concentration varies between 1.5 and 3 mM and increases in direct relation to the percentage of slow fibres (Vandenborne et al., 1995). Thus Pi/PCr ratio is higher in slow than in fast muscle fibres. Increase of Pi concentration up 8-fold has been observed with NMR during exercise (Vandenborne et al., 1991).

The *rate of energy consumption* in muscle fibres is very low at rest and increases to its maximum during contractile activity. As shown by Stienen et al. (1995) the total rate of energy consumption can be estimated from the sum of the ATP consumed by myofibrillar ATPase and ATP consumed by ionic transport. Using the values measured during maximal activations in human single demembranated fibres (Stienen et al., 1996) the following values of myofibrillar ATP consumption rate in physiological conditions have been re-calculated by Sahlin: type I: 6.5 mmol/kg dw/s, type IIA: 17.6 mmol/kg dw/s, type IIX: 26.6 mmol/kg dw/s (Sahlin et al., 1998). These values should be increased by 25–40% to account for ionic transport in intact fibres (Stienen et al., 1995). Direct measurements of ATP consumption in vivo during contractile activity have been performed using NMR techniques or single fibres microchemical methods. Greenhaff et al. (1993) have determined ATP consumption rates of 12 mmol/kg dw/s in fast fibres and 6.1 in slow fibres during maximal isometric contractions induced with electrical stimulations of human quadriceps with blood flow occluded. These values (which correspond to 3 and 1.5 mM/s assuming dry/wet weight ratio = 0.25) are fully compatible with in vitro single fibres measurements and also with NMR determinations. Using NMR, Turner et al. (1993) have calculated a ATP consumption rate of 4.4 mM/s during maximal isometric contraction of human adductor pollicis and 9.7 mM/s during shortening against a load corresponding to 30% of isometric tension. Lower values [0.15 mM/s (Blei et al., 1993) and 0.5–1 mM/s (Boska, 1991)] have been obtained during submaximal contractions. Resting ATP consumption rate is more than 100 times lower: for example Blei et al. (1993) have calculated 0.008 mM/s.

The energy balance of the muscle fibres requires that ATP consumption rate is compensated by *ATP re-synthesis rate*. The first and faster reaction is catalyzed by creatine kinase which allows conversion of ADP to ATP spending energy stored in PCr. As a consequence of this at fatigue and after strenuous exercise PCr content, which is higher in fast than in slow fibres, decreases to low values in both fast and slow fibres and Pi increases (see above). At variance with other enzymatic activities creatine kinase shows little variations among fibres in human muscles (Lowry et al., 1978).

Glycolytic and oxidative ATP synthesis allows *PCr recovery* after exercise. PCr concentration increases after the end of the contractile activity: the recovery is faster in slow than in fast fibres during the first minutes, then becomes similar. In the first minute after maximal exercise the rate of recovery is higher in slow fibres than in fast fibres (Soderlund and

Hultman, 1991). Later on the rate decreases in slow fibres whereas it remains higher in fast fibres leading eventually to the rest PCr concentrations. A transitory post-exercise increase of PCr content has been observed in fast fibres (Soderlund and Hultman, 1991) and in slow fibres (Sahlin et al., 1997).

Activated by the increase of AMP and IMP concentrations, *glycogenolysis* promotes the PCr resynthesis based on the glycolytic pathway (Sahlin et al., 1990). The rate of glycogen breakdown (expressed in glucosyl units) in human muscles in vivo has been estimated to be 1.9–3.4 mmol/kg dw/s (Chasiotis, 1983). A peak of 7.5 mmol/kg dw/s has been measured in the first 15 s of high intensity exercise in human gastrocnemius (Walter et al., 1999). Single fibre determinations during maximal contractions elicited with electrical stimulation show a clear cut difference between slow and fast fibres: 0.18 mmol/kg dw/s in slow fibres vs 3.54 mmol/kg dw/s in fast fibres (Greenhaff et al., 1993). The large difference is due partly to the higher phosphorylase activity and partly to the higher increase of AMP and IMP concentration in fast fibres. When circulation is occluded during exercise, phosphorylase activity can be further stimulated reaching 2.05 mmol/kg dw/s in slow fibres and 4.32 mmol/kg dw/s in fast fibres (Greenhaff et al., 1993). Under these conditions the difference between fast and slow fibres is reduced: the values of phosphorylase activity are close to V_{max} of phosphorylase and suggest that the glycogen breakdown is maximally activated. The rate of the glycolytic pathway is about 30% lower than the rate of the glycogenolysis: a rate of lactate accumulation of 2.43 mM/s has been determined by Walter et al. (1999). In accordance with the large difference in the rates of glycogenolysis all enzymes of the glycolytic pathway exhibit pronounced variations (up to 9–10 times) between fast and slow fibres (Lowry et al., 1978).

The pattern glycogen depletion in human muscles is highly selective: first glycogen decreases in type I and type IIA fibres and then at the end of the exercise a partial depletion occurs in type IIX fibres (Gollnick et al., 1974; Vollestad et al., 1984). This probably reflects the recruitment sequence: the more oxidative fibres are recruited during the whole exercise period, whereas fast IIX only towards the end.

Glycogen resynthesis which follows glycogen depletion occurs initially faster in slow than in fast fibres; only after 3 hours does the rate decline in slow fibres whereas it remains higher in fast fibres until final resting values are reached (Casey et al., 1995).

The potential for ATP re-synthesis via *aerobic oxidative processes* is 2–3-fold lower than the potential of anaerobic generation (Blomstrand et al., 1986, 1997; Greenhaff et al., 1993; Walter et al., 1999). It can guarantee, however, a high capacity ATP provision during prolonged exercise. The maximal rate of oxygen consumption is 353 ml/kg ww/min (i.e. 0.2 mM/s) and this corresponds to a rate of the Krebs cycle of 0.076 mM/s or 4.6 mmol/kg ww/min (Blomstrand et al., 1997). Interestingly, this corresponds to the maximal oxoglutarate dehydrogenase activity (4.3 mmol/kg ww/min = 0.07 mM/s) and is not very different also from maximal SDH activity (Blomstrand et al., 1997). These enzymes represent therefore the rate limiting steps of the cycle whereas other enzymes as citrate synthase have higher maximal activities (Blomstrand et al., 1997). From the rate of Krebs cycle assuming that 18 molecules of ATP are produced from the oxidation of each acetate the maximal rate of aerobic ATP generation can be calculated to be 1.37 mM/s or 5.5 mmol/kg dw/s. Aerobic oxidative ATP generation also reveals significant differences between human skeletal muscle fibres. The detailed analysis of enzymatic activities in human single fibres carried out by Essen-Gustavsson

and Henriksson (1984) show that citric cycle enzyme activity is 35% higher in type I than type II fibres of human muscles. This is in agreement with previous results obtained by Lowry et al. (1978) (30% higher) and Essen et al. (1975) (55% higher). Whereas clear differences are detectable between slow and fast fibres, the differences between the two types (IIA and IIX) of fast fibres seem rather small and a large overlap is present (Reichman and Pette, 1982; Essen-Gustavsson and Henriksson, 1984).

It can be of interest to calculate the relative contribution of anaerobic and aerobic processes to the maximal rate of ATP generation in human muscles. Table 5 reports the results of the calculation done on in vitro measurements by Blomstrand et al. (1986) and of a calculation done compiling data obtained in vivo by Blomstrand et al. (1997) and by Greenhaff et al. (1993). As can be seen anaerobic power represents 50–90% of the total metabolic power, being higher in untrained subjects (who very probably possess a greater proportion of fast fibres) and in fast fibres. It is worth to observe that aerobic power of ATP generation in slow fibres precisely matches the ATP consumption of slow fibres during maximal isometric contraction (6 mmol/kg dw/s) as calculated by Sahlin et al. (1998) on data by Stienen et al. (1996a) (see above). On the other hand ATP consumption of fast fibres during maximal isometric contraction (17.6–26.6 mmol/kg dw/s, see above) is greater than the metabolic power of fast fibres. This implies a consumption of PCr without resynthesis during contractile activity. The facts that fibres are very seldom maximally activated and that contraction is generally associated with movement represent obvious limits of these calculations.

4. Optimization of muscle fibre function in vivo: a working hypothesis

The large heterogeneity in functional properties of human muscle fibre types coupled with their uneven distribution in different human muscles is very probably a major determinant of the remarkable capacity of human skeletal muscles to perform extremely variable motor tasks.

Table 5
Maximal rate of ATP generation in vitro and in vivo via anaerobic and aerobic pathways

		Total	Anaerobic	%	Aerobic	%
25° in vitro data from Blomstrand et al. (1986)						
Trained men	mM/min	98	72	73	26	27
	mM/s	1.6	1.2		0.4	
Untrained men	mM/min	117	104	88	13	12
	mM/s	1.9	1.7		0.2	
37° in vivo compiled from Blomstrand et al. (1997) and Greenhaff et al. (1993) and assuming that Krebs cycle rate is 35% higher in slow fibres and that muscles are composed by 50% fast and 50% slow fibres						
Fast fibres	mM/s	4.15	3	72	1.15	28
	mmol/kg dw/s	16.6	12		4.6	
Slow fibres	mM/s	3	1.5	50	1.5	50
	mmol/kg dw/s	12	6		6	

Slow fibres appear particularly suited for isometric, tonic contractions as they develop almost the same isometric force as fast fibres, but consume much less ATP (low tension cost, Fig. 7B) and fatigue very little due to their mostly aerobic metabolism. Developing very low power, they are unlikely to support very powerful movements. However, developing their maximum power with their highest efficiency at low velocity (low V_{opt}), they are particularly suited to support slow movements (Figs. 4C and 11). On the contrary, IIX fibres, having much higher power and developing their maximum power with their highest efficiency at high velocity, are much better used for fast and powerful movements. Moreover, their low aerobic metabolism and high anaerobic power suggest they cannot support long lasting contractions, but only short burst of activity. Type IIA fibres which are the most abundant fast fibres and have intermediate optimal velocity, power and tension cost, are more conveniently used for a wide range of movements at intermediate speeds.

Muscles more frequently involved in maintaining posture have higher relative content in slow fibres: for example 70–80% slow fibres in soleus versus 30–35% slow fibres in triceps and 30–40% slow fibres in biceps brachii (Saltin and Gollnick, 1983; Klitgaard et al., 1990; Harridge et al., 1996). Athletes involved in sprint running have higher relative content in fast IIA and IIX fibres than athletes involved in the marathon (Saltin and Gollnick, 1983). These data support the idea that slow fibres are used for posture control and for slow and repeated contractions whereas fast fibres are involved in phasic, powerful and short exercise bursts.

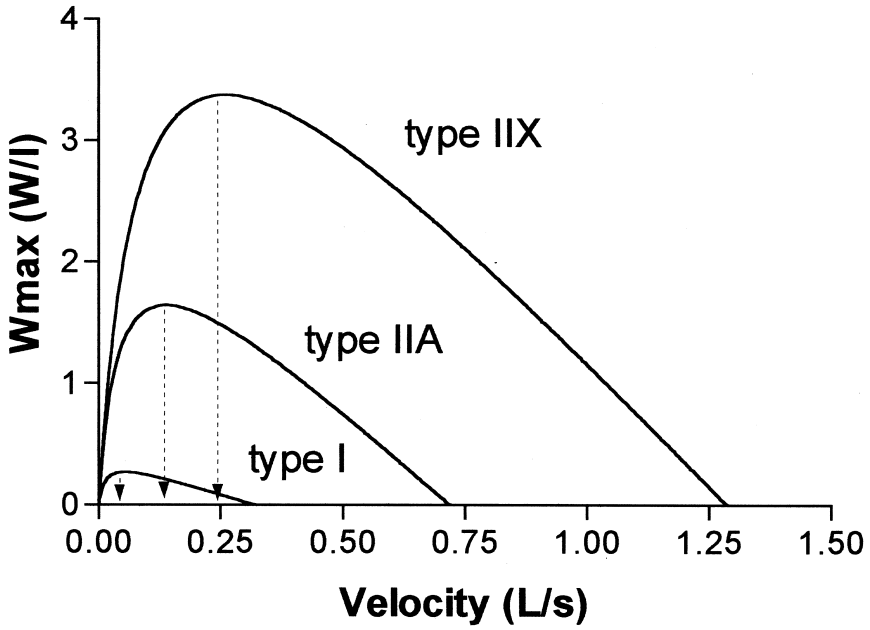

Fig. 11. Velocity–power curves of representative type I, type IIA and type IIX human skeletal muscle fibres. Arrows indicate V_{opt} for type I, IIA and IIX fibres. Power–velocity curves are calculated from the parameters of Hill's hyperbolic equation used to fit experimental force–velocity curves. Power is expressed in Watt per liter (W/l). Fibres are classified on the basis of MHC isoform composition determined by SDS-PAGE. Figure redrawn and modified from Bottinelli et al. (1996).

Interestingly, in this regard, electromiographic determination of muscle usage in vivo during everyday activity suggests that average percentage of time active is highly correlated with the percentage of type I fibres of a muscle (Monster et al., 1978). A direct demonstration of the energy-saving effect of slow fibre recruitment has been recently given by Ratkevicius et al. (1998). In this study the ATP cost of submaximal force development has been found much lower (ca 50%) in voluntary than in electrically induced contractions. This has been explained assuming that electrical stimulation activates both slow and fast fibres, whereas slow fibres are preferentially activated in voluntary contractions. Finally, some correspondence between fibre type composition and in vivo functional properties of skeletal muscles has been found (Thorstensson et al., 1976; Gregor et al., 1979; Harridge et al., 1996).

This notwithstanding, a comprehensive picture of how the heterogeneity in the properties studied in vitro can actually determine the remarkable capacity of human skeletal muscle to perform very different motoric tasks in vivo is still lacking. This is mainly due to the complex control of in vivo muscle performance. Muscle performance in vivo, in fact, is determined not only by fibre type composition, but also by neural control and by other factors such as muscle architecture (Narici et al., 1996). Only a working hypothesis of how some aspects of human skeletal muscle function like fatigue, power, and efficiency can be optimized taking advantage of the existence of fibre types with different properties can be put forward. Such hypothesis is based on existing models for more simple animals (Rome et al., 1988; Lutz and Rome, 1994) and on information on motor unit recruitment in humans accumulated since the classical work by Henneman et al. (1974).

Since the elegant studies by Rome and coworkers in fish (Rome et al., 1988) and frog (Lutz and Rome, 1994) muscles, W_{max} and V_{opt} have been considered key characteristics of muscle contraction in vivo. Combining in vivo and in vitro measurements, Rome et al. (1988) have demonstrated that in vivo in the carp: (1) slow and fast muscles shorten at different velocities, but both shorten at optimal velocity, i.e. at the velocity at which their muscle fibres in vitro develop maximum power; (2) either slow or both slow and fast fibres, in fact, are selectively recruited to allow movement in the whole range of physiological speeds and, at the same time, to optimize mechanical power at all speeds. In humans, combined in vitro and in vivo measurements of shortening velocity and power of muscle fibres and determination of fibre type recruitment during movement have not been feasible so far. Fish is an ideal system to perform such kind of studies as fish muscles are homogeneously slow or fast, are selectively recruited to perform very different motor tasks, their electrical activity and their shortening velocity can be determined in vivo, and finally contractile properties of their muscle fibres can be studied in vitro. Each human muscle is a mixed muscle which generally performs different motoric tasks. Motor units in humans are recruited according to a complex pattern which is difficult to study in detail during movements at different speeds. Technical problems make it difficult to assess shortening velocity of muscle fibres in vivo. However, the 7-fold range in optimal velocity of human fibres (Fig. 4C) suggests that, in human muscles, fibres are available to allow optimization of power and efficiency at very variable speeds. As each fibre type covers a distinct portion of the whole range of variability of V_{opt}, during movement and locomotion at increasing speeds of movements power out-put could be optimized recruiting human muscle fibres in the order I > IIA > IIX. Interestingly, even during the fastest movements when, in keeping with this hypothesis, IIX fibres would shorten at their V_{opt}, slow fibres, although

contributing little to power, would still be able to shorten and at least would not oppose shortening (Fig. 11).

That type I, IIA and IIX fibres in humans can actually be recruited in such a way to optimize power and efficiency is suggested by the classical studies by Henneman of motor unit recruitment. According to Henneman's size principle (Henneman et al., 1974; Bawa et al., 1984), motor units are recruited in a stereotyped way: slow, fatigue resistant, motor units (S) are recruited for movements that require low speed and low force (standing and walking), fatigue resistant (FR) motor units are recruited for movements requiring higher speed and force (running), finally fast fatigue (FF) motor units are recruited for the fastest and strongest movements (jumping). As S motor units are the least fatiguable and the weakest and FF the most fatiguable and the strongest, the size principle has long been considered a way to allow a smooth accumulation of force during contraction and to optimize fatigue. Interestingly, as S motor units are made of type I fibres, FR motor units of type IIA fibres and FF motor units of type IIX fibres, a recruitment order according to the size principle would at the same time optimize also power and efficiency.

Whether the size principle holds in all conditions and especially whether it holds in humans is still largely debated (Desmedt and Godaux, 1977; Kanda et al., 1977; Thomas et al., 1978; Nardone et al., 1989). Significant modifications in the size principle have been suggested. The idea that motor units can be organized into groups recruited in order during a motoric task, i.e. into task groups, has been introduced (Chanaud et al., 1991). It has also been suggested that a task group does not necessarily correspond to a muscle, can dynamically change for subtle changes in the motoric task, and can even span different muscles in the same group (Calancie and Bawa, 1990; Cope and Pinter, 1995). Task groups, therefore, can account for the observation that within a muscle FR or FF, motor units can be recruited and small, S, motor units can remain silent. The size principle for many authors means that within a defined task group, if not within a muscle, motor units are recruited in the order S > FR > FF. Finally, true exceptions to the size principle not accounted for by the existence of task groups have been found during eccentric contractions (Nardone et al., 1989).

It should also be pointed out that cautions must be taken when attempting to transfer information collected at low temperature in vitro in isolated skinned fibres to in vivo conditions. In vitro studies, as shown above, safely allow us to compare properties of different fibre types. However, due to the effect of temperature on contractile and energetic properties (Fig. 5), they do not allow us to gather precise information on the absolute values of force, velocity, power and possibly efficiency of human fibres at physiological temperatures in vivo. It should also be mentioned that temperature has been found to have a different impact on functional properties of slow and fast muscles at least in some temperature ranges (Ranatunga, 1982, 1984). Should this be the case for isolated fibres, increasing temperature might alter the observed differences among fibre types and not only the absolute values of the parameters studied.

5. Conclusions

As the evidence reviewed here demonstrates, the muscle fibres of human skeletal muscles are

very heterogeneous in their functional and structural properties. This heterogeneity covers all possible aspects of muscle contractile function and is directed in optimizing the contractile responses and in performing different motor tasks minimizing fatigue. However, although the above-reported working hypothesis can be profitably used to interpret differences in whole muscle function in vivo, a comprehensive picture of how muscle fibre heterogeneity is actually used in vivo is still lacking.

The functional and molecular heterogeneity present in human muscle appears certainly different from that described in the muscles of small mammals such as rat or rabbit. Some of the results discussed above might suggest that in human muscles diversity is reduced compared to other mammalian species. For example, two MHC isoforms (MHC-IIB and MHC-m) are not expressed and less tight rules regulate preferential associations in isoform expression. However, the functional analysis shows that the range of variation of parameters as V_o, V_{opt}, W_{max} is not more narrow in humans than in other mammalian species. If anything, the range of variability of V_o is larger in humans (7–8-fold) than in rat (3–4-fold) or rabbit (4–5-fold). In other words the same range of variation is covered with the available isoforms (MHC-I, MHC-IIA and MHC-IIX) and fibre types (I, IIA and IIX) and no functional diversity is lost. This implies that the diversity among the MHC isoforms expressed is larger in man than in rat or rabbit.

The heterogeneity produced by myofibrillar proteins is extremely large, even larger than the heterogeneity produced by metabolic enzyme diversity. During maximal activations myosin isoforms can modulate mechanical power over a range of variation of 10 times and ATP consumption over a range of 7 times. On the other hand the rate of maximally activated glycolytic processes varies over a range of only 2 times and even less is the range of variations of aerobic oxidative energy production. Thus the most effective regulation of contractile performance and resistance to fatigue seems to be the energy consumption rather than the energy production.

Many questions still await an answer. Among them not only the matching between fibre properties and their control by nervous system, but also whether this matching has features specific for human beings. The lack of detailed studies on muscle fibres of large mammals and on muscle fibres of mammals at least partially comparable with humans as motor control and locomotion (like primates) makes a definitive conclusion impossible. More work is necessary to assess how unique for humans is the picture of muscle fibre heterogeneity which emerges from this study.

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