Cellular and Molecular Basis of Heterogeneity in Contractile Performance of Human Muscles

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Abstract
Human skeletal muscles are able to fulfil very different mechanical tasks. The basis of this ability can be found in the existence of different fibre types which can be activated selectively by the nervous system in relation to the required performance. The study of single muscle fibres dissected from biopsy samples has allowed to characterize contractile performance in relation to molecular composition. In particular the presence of distinct myosin isoforms dictates the rate of ATP hydrolysis and the shortening velocity, whereas the presence of specific isoforms of regulatory proteins modulates the ability to respond to activator calcium. Proteins of the sarcoplasmic reticulum and of the plasma membrane determine the excitability and duration of the contractile cycle (contraction speed). Abundance of mitochondria and activity of enzymes of the glycolytic pathway determine the metabolic power of the fibres and from the balance between rate of energy consumption and rate of energy production the resistance to fatigue is determined. In this short review the major determinants of the contractile and energetic properties of human fibres will be considered and the way the nervous system can exploit them to optimize the contractile performance will be discussed.
Key words: contraction speed, heterogeneity of contractile performance, human muscles, mechanical power, molecular basis, resistance to fatigue.

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Introduction

Skeletal muscles are living engines which produce mechanical energy starting from chemical energy, and myosin is the molecular motor responsible for this energy transduction.

Skeletal muscles can be employed in a large variety of mechanical tasks. As a general rule different muscles in the body fulfil specific tasks: for example, the contraction of the wrist flexors and the finger flexor support the light and fast touch of the pianist finger on the keyboard, whereas the strong leg quadriceps extend the leg when the football player kicks the ball more than fifty metres away. In some cases, however, the same muscle can also be employed in extremely different tasks: for example, a muscle such as adductor pollicis can be used to thread a fine needle as well as to grasp a bar and contribute to support the whole body weight. Finally, when the performance of corresponding muscles is compared in different animal species, the diversity of functional tasks is surprising: in all mammals the soleus and the gastrocnemius muscles work to extend the ankle and support the body weight in static and dynamic conditions, even though the body weight can vary by more than ten thousand times if, for example, a mouse and a cow are compared.

The control of muscle performance is in the hand of the nervous system: at each time during a movement the motor centres decide how many motor units must be activated and at which frequency of action potential discharge. Inside each motor unit the force developed increases in relation with discharge rate (force-frequency relation) and inside a muscle the higher the number of motor units activated the greater the force. The regulation, however, is not only quantitative. With the exception of some muscles which are composed of only one type of motor units, the majority of muscles consist of motor units with different properties: force development, power output, time course of contractile responses, resistance to fatigue all vary from one motor unit to the other. Thus, through a selective activation, or to use a more specific word 'recruitment' of motor units with the more appropriate properties, a given muscle can behave in different ways in relation to the functional task that is required.

The diversity among motor units, discovered in the sixties [13, 32] yields to identify at least three main types:

- motor units S or slow which develop little force, with slow time course and are very resistant to fatigue
- motor units FF or fast fatiguing which develop high force with a fast time course but undergo quickly to fatigue
- motor units FR or fatigue resistant which are intermediate between the other two groups as force development and fatigue resistance.

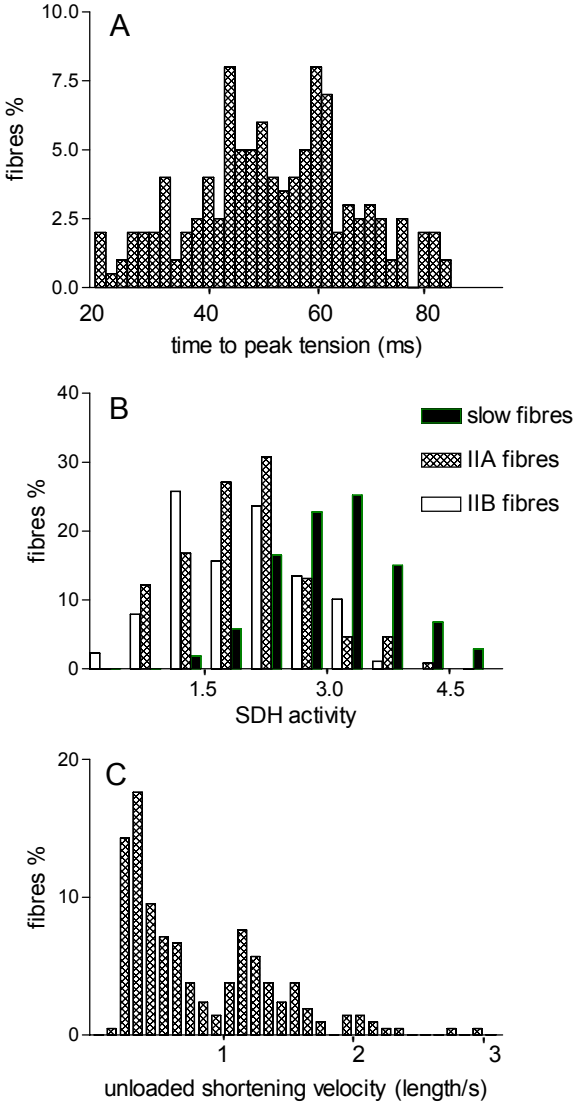

Figure 1: Examples of diversity among human muscle fibres. A. Distribution of the contraction times (time from stimulus to peak tension) of fibre bundles from biceps brachii. B. Distribution of SDH activity of single fibres from tibialis anterior: fibres are divided in three groups according to their ATPase activity. C. Distribution of maximum shortening velocity of single fibres from leg muscles (vastus lateralis, soleus and tibialis anterior): the three peaks probably correspond to slow, fast IIA and fast IIX fibres. From Bottinelli & Reggiani 2000.

The basis of this diversity is localized essentially in the muscle fibres composing the motor unit: muscle fibres are different from each other.

When observing the muscle fine structure using electron microscopy, the similarity between muscle fibres in all animal species is remarkable: the sarcomeric structure appears highly conserved in all muscle fibres of all vertebrates. The same holds true for the molecular architectures of the sarcomeric muscles: all muscle fibres of all animal species are virtually composed of the same proteins. Only some quantitative features, for example fibre thickness, vary in an obvious way. In contrast, when muscle fibres are dissected free from the muscles and their functional properties analysed, a large diversity can be observed: individual muscle fibres differ for their mechanical power output, speed of shortening and resistance to fatigue in prolonged contractile activity.

Which then is the basis of this functional diversity ? In spite of the structural homogeneity, muscle fibres become functionally heterogeneous as they are composed of different isoforms of the same proteins. With the term isoforms we define two or more proteins which have only slight differences in amino acid sequence, however these small differences determine clearly distinct functional properties. Virtually all muscle proteins can exist in two or more isoforms: extreme examples are represented by troponin T fast which in each mammalian species may be produced in 64 isoforms obtained by alternative splicing of the same gene [8-9] and myosin, which is an hexamere (composed by two myosin heavy chains or MHC and four myosin light chain or MLC) which may be produced in at least 60 isoforms [45].

In the following sections we will briefly review the variability of contractile properties among muscle fibres and delineate their molecular basis. Also there will be a brief look at how the nervous system can exploit this variability during motor performance and how nervous stimulation, mechanical load and hormonal factors can modulate the fibre heterogeneity over long term.

Functional heterogeneity and molecular heterogeneity of muscle fibres

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: three examples of which are reported in Figure 1. The first parameter – time to peak tension (panel A)– is dependent on the rate of calcium release and reuptake by the sarcoplasmic reticulum, and on the rate of force generation during actin-myosin interaction. The second parameter – SDH activity (panel B) – is an enzyme activity directly related to the production of ATP. The third parameter – maximum shortening velocity (panel C) – is determined

by the rate of actin-myosin interaction and ATP hydrolysis, and is one major determinant of the ability to generate mechanical power.

As Figure 1 suggests, the molecular heterogeneity implicated in the functional heterogeneity extends from intracellular calcium kinetics to biochemical pathways for energy production and chemo-mechanical energy conversion in the myofibrils. The molecular diversity is based on gene regulation through two main mechanisms:

1) Qualitative mechanisms: as stated above, many muscle proteins exist as 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 mechanisms: differential expression of the same gene. Many genes can be up- and down-regulated independently of each other, based on 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 mechanisms described above 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 reduce and some more frequent phenotypes of muscle fibres appear. The identification of these more frequent phenotypes, generally indicated as fibre types, becomes a preliminary step to study muscle fibre diversity.

The fact that distinct fibre types do exist is best demonstrated by the conditions when fibre transformations take place. In the first place during prenatal development, fibre types emerge during the three waves of myoblast fusion typical of human muscles [22]. Until the 20^{th} week, there is no detectable difference among fibres, later on, around one year postnatal, when all fibres are fully differentiated, the adult phenotypes can be identified by their specific ATPase staining and enzyme histochemistry. In addition, the fibre phenotype can be further altered during postnatal life 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 muscle fibres move from one phenotype to the other (fibre 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 greater detail in animals, particularly in small mammals such as rat or rabbit and have been recently well reviewed by Pette and Staron [45]. Several data are, however, also available on muscle fibre plasticity in humans (for example induced by training or by disuse) and substantially confirm what is already known from animal studies.

Functional characterization of human muscle fibre types: contractile properties

As mentioned above, motor units differ in their contractile properties: some are stronger and faster in their contraction and some are weaker and slower. This property of motor units reflect rather closely the properties of the muscle fibres which belong to the motor unit and can be studied *in vitro* after dissection. Needle or surgical biopsies provide the tissue sample from which single muscle fibres can be dissected. The biopsy sample is immersed in a skinning solution containing a high concentration of potassium propionate and EGTA, and is divided in small bundles [7]. From these bundles segments of single fibres are dissected under a stereo-microscope, the membranes are permeabilised with a detergent (for example Triton X100) and the fibres are mounted in the experimental apparatus for *in vitro* determination of contractile and energetic properties. Fibres are activated directly with appropriate calcium concentration (pCa=4.5) and develop tension or shorten consuming ATP as energy source. Once the functional analysis is done, single fibres are tested by SDS-PAGE (and in some cases Western Blot) for the MHC isoform composition. Myosin heavy chain isoforms are preferably used as molecular markers of the fibres types. The choice of MHC isoforms to classify skeletal muscle fibres have several reasons: MHCs are the most abundant proteins of muscle fibres and are the major determinant of both ATP consumption rate and mechanical performance (for example, mechanical power output). They are also responsible for the sensitivity of myosin ATPase to alkali or acid preincubation, the feature generally used for fibre typing in muscle histochemistry in neurological diagnostic protocols [11, 29]. If MHC isoforms are adopted to classify fibres, groups with specific functional characteristics can be delineated. In adult human muscles five types of fibres can be identified on the basis of their MHC isoform composition: slow, fast IIA, fast IIB or IIX, mixed I-IIA, mixed IIA-IIX. As shown by Smerdu et al. [57] the human fibres which were previously indicated as IIB, contain a myosin isoform very similar to the isoform IIX expressed in other animal species: they are therefore more correctly called IIX fibres.

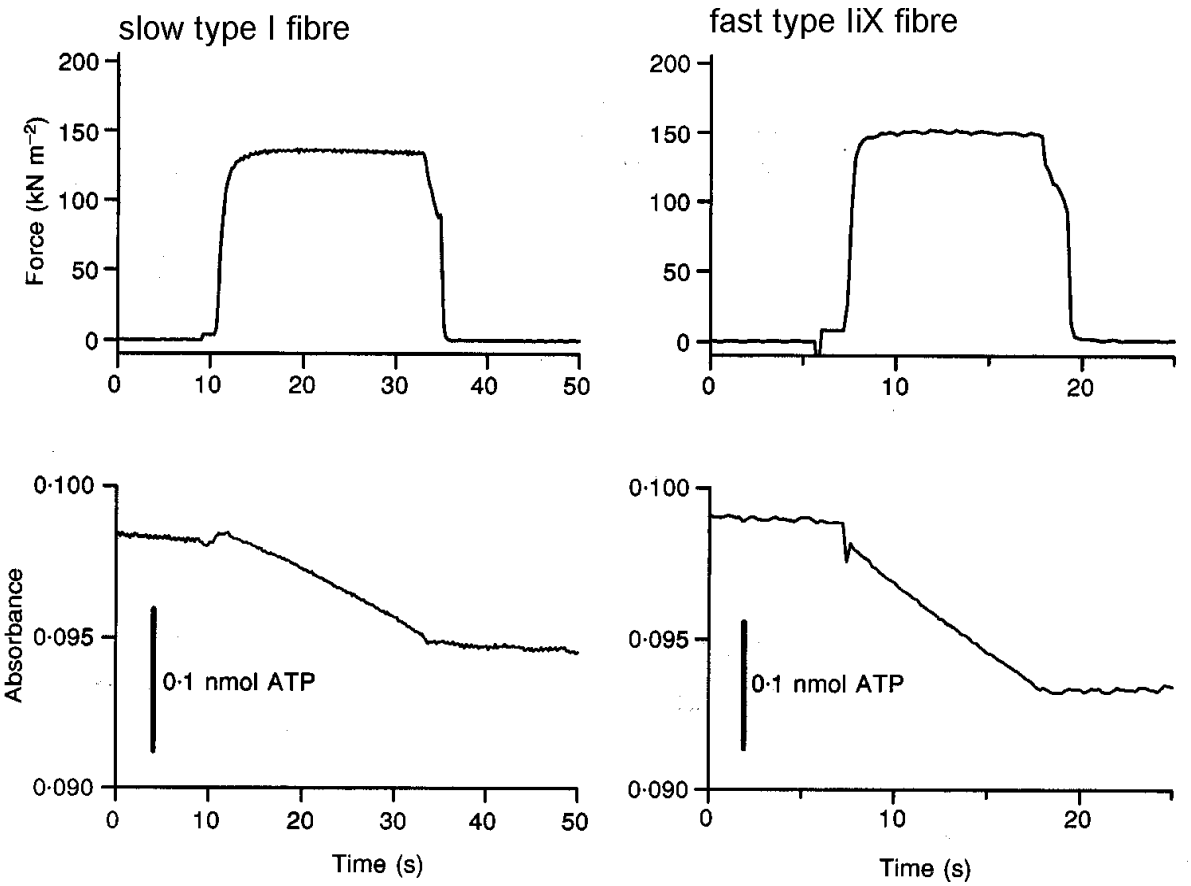

Figure 2: Experimental recordings of tension development and ATP consumption during isometric contractions of slow and fast fibres (modified, from Stienen et al 1996).

Figure 2 shows tracings of isometric contractions of a fast and a slow single fibre in vitro and the simultaneous recording of ATP consumption. Average values of the isometric tension (Po) and ATP consumption rate (Ao) are reported in Table 1. Fast fibres hydrolyse ATP faster than slow fibres and, among fast fibres, IIX fibres consume more ATP than IIA fibres [30, 58].

If during contraction fibres are allowed to shorten against various loads the force-velocity curve can be obtained. From force and velocity the mechanical power developed by the fibre can easily be calculated. Representative force-velocity curves and power-velocity curves of the three main fibres types are displayed in Figure 3. The diversity in maximum shortening velocity can reach as much as 4 times and the difference in peak power can be even higher [7, 37].

Several studies [3-7] have shown that the values of ATP consumption rate during isometric contraction as well as of maximum shortening velocity and peak power during active shortening are directly determined by the isoform of MHC present in the muscle fibre.

Recently a protocol to extract and purify tiny amount of pure myosin isoforms from single fibres has been developed [16]. The speed of actin filament translocation in in vitro motility assay has been determined for various myosin isoforms extracted from single human fibres (Canepari, Bottinelli and Reggiani, unpublished observations). As shown in Figure 4, the speed of actin filament translocation (Vf) on a surface covered with pure myosin isoforms varies in close proportion with the maximum shortening velocity (Vo) of single fibres containing the same myosin isoforms. This correlation strongly supports the view that myosin isoforms are the only determinant of maximum shortening velocity in human fibres.

The amount of force developed during contraction in isometric contraction is proportional to the concentration of calcium in the medium perfusing the fibres. Figure 5 shows the pCa-tension curves, which are used to describe the sensitivity of the contractile system to activator calcium: differences both in slope (less steep in type I than in type II fibres) and in position along the abscissa are visible.

The isoforms of regulatory proteins (tropomyosin, troponin C, T, I) present in the fibre are the determinant of the slope and the position of the pCa-tension curve [5,19, 56].

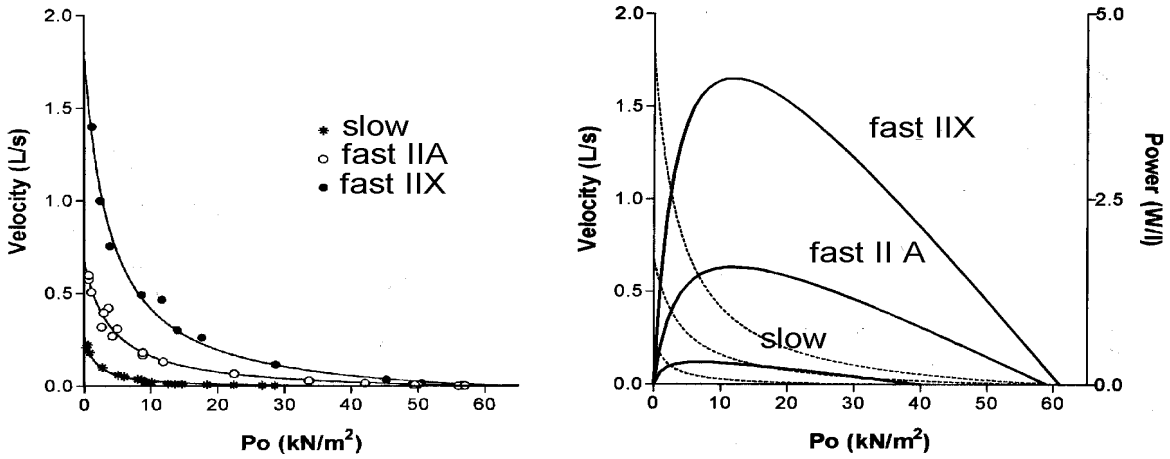

FIGURE 3: Force-velocity and power-velocity curves of the three main fibre types in human muscles, determined in vitro at 12°C.

Heterogeneity of electrophysiological properties and excitation-contraction coupling in human muscle fibres

The speed of the contractile cycle is markedly different among fibres and motor units and this difference (and not the diversity in maximum shortening velocity) gives origin to the notion of slow and fast motor units (see above): the time to peak tension shown in Figure 1A varies from 20 to 80 ms in human biceps brachii. The contractile speed is directly related with the major calcium regulating membrane system, the sarcoplasmic reticulum. Calcium is released faster and in larger amount and is also taken up more efficiently in fast than in slow fibres. Direct evidence that calcium transients are different in slow and fast fibres comes from studies on animal muscle fibres with fluorescent indicators [25,27]. 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 human fibres [20]. The molecular basis of the diversity lies firstly in the fractional volume of the sarcoplasmic reticulum: an inverse relation between volume of the terminal cisternae and time to peak tension has been shown by Kugelberg and Thornell [36]. Two isoforms of the calcium pump are present: SERCA1 typical of fast fibres and SERCA2 typical of slow [39]. The density of the calcium pump is higher in fast than in slow fibres [26] and differences are also present between fast IIX and fast IIA fibres [55].

In relation to the different speed of the contractile cycle and the resistance to fatigue the discharge rate of the motor neurons is much higher in fast than in slow motor units: chronic motor unit recordings show that slow motor units are active for longer times at low frequency whereas fast motor units are active in bursts of short duration and higher frequency [33,40] in rat muscles). Thus, in addition to the other aspects of heterogeneity in contractile response described in the

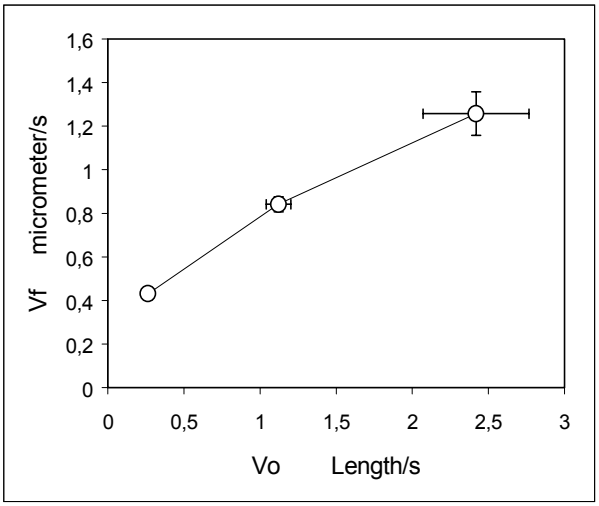

FIGURE 4: Relation between speed of actin filament translocation on pure human myosin isoforms in in vitro motility assay (Vf) and maximum shortening velocity of single fibres containing the same myosin isoforms (Vo).

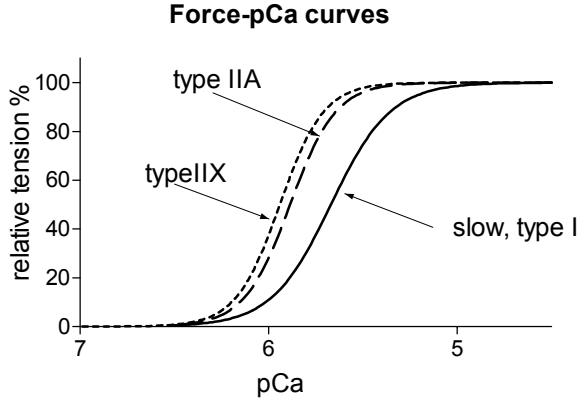

FIGURE 5: pCa-tension curves of the main three fibre types of human muscles determined in vitro

previous sections, muscle fibres are different in their excitability and ability to follow the rate of motor neuron discharge. 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 despite the accumulation of potassium in extracellular space and particularly in T tubules. In contrast, fast fibres (among them IIX fibres to a greater extent than IIA fibres), need to quickly recover excitability 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 [2,32,40].

In human muscles the resting potential does not show significant differences among the two fast fibre types, but is less negative in slow fibres [50, 51, 53]. The membrane capacitance and the membrane resistance per unit area seem to be similar in both fast and slow fibres [50]. Whereas in neurons the membrane ionic conductance is dominated by potassium, in muscle fibres 70% of the membrane conductance is due to chloride ions [10] and only 30% to potassium. There are at least two isoforms of *chloride channels* expressed in muscles: the voltage gated ClC1, which is muscle specific and important for fibre repolarisation after action potential, and the ubiquitous ClC2 [47]. There is no available evidence of heterogeneous expression in different fibre types.

Na channels play a central role in determining excitability of muscle fibres. Excitability of the membrane is determined by the number of Na channels and the fraction of inactivated channels. The density of Na channels is greater in fast than in slow fibres in humans [50] and other mammals [15]. In each fibre, Na current density is higher at the border of the endplate than away from it [52], thus ensuring that an action potential is triggered every time acetylcholine release produces an end plate potential. This decrease in Na current moving away from the endplate is reported as being much lower in slow than fast fibres [50]. Even though no local differences (close and away from the endplate) have been found in the voltage dependence of the activation and inactivation processes [53], differences are present in the inactivation process between slow and fast fibres [51]. These differences may be relevant for keeping fibre excitability during repetitive action potential firing.

Metabolic heterogeneity of human muscle fibres

One of the most notable diversities between single muscle fibres deals with fatigue resistence. Motor units are divided in fatiguable and fatigue resistant and this property depends on muscle fibre characteristics. Muscle fatigue can be defined as a decrease of force development which follows repeated contractions. The decrease in force is determined by the accumulation of by products of chemical energy supply: inorganic phosphate, lactate, hydrogen ions [60].

The energy necessary for the contractile activity is provided by ATP hydrolysis to ADP and Pi. ADP is then re-synthetised to ATP from PCr through the creatin kinase reaction. PCr represents the molecular form to store chemical energy and is produced from creatine and ATP. ATP in turn is synthetised 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 and ketone bodies provides a supply of acetyl-CoA which is the substrate for the mitochondrial oxidative processes. During contractile activity, lactate, Pi and hydrogen ions may accumulate in muscle fibres triggering the decrease in contractile performance called fatigue.

Muscle fibres can resist better to fatigue if they can re-synthesise promptly ATP and if they can avoid lactate accumulation with a fast catabolism of pyruvate to lactate and then to water and carbon dioxide through mitochondrial oxidative processes. In simplified terms resistance to fatigue can be associated with active mitochondrial energy production.

The metabolic heterogeneity of muscle fibres was first observed with histochemical determination in muscle cryosections of the enzyme activities of glycolytic (LDH) and mitochondrial oxidative (SDH, COX, NADH) processes [43, 46]. In the muscles of small mammals three main fibre types were classified: S or slow-twitch oxidative and FOG or fast twitch oxidative-glycolytic (both based on enzyme activities of aerobic substrate oxidation), and FG or fast-twitch glycolytic [1, 44]. Combining single motor unit stimulation with histochemical analysis of enzymatic activity it was found that: 1) the fibres belonging to the same motor unit have identical metabolic properties and, 2) enzymatic properties of the fibres can be correlated with resistance to fatigue: slow fatigue-resistant motor units are composed of SO fibres, fast fatigue-resistant and fast fatigable motor units, are composed of FOG and FG fibres respectively [12, 13, 42].

Microchemical methods allow determination of substrate concentrations and enzyme activities in single human muscle fibres [23, 24, 38, 48]. 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 [48] (see Figure 1B). Determination of more than one enzymatic activity in the same fibres reveals direct correlations between enzymatic activities of the same pathways and inverse correlations between glycolytic and aerobic-oxidative enzymatic activities (see for example Figure 6, [49]).

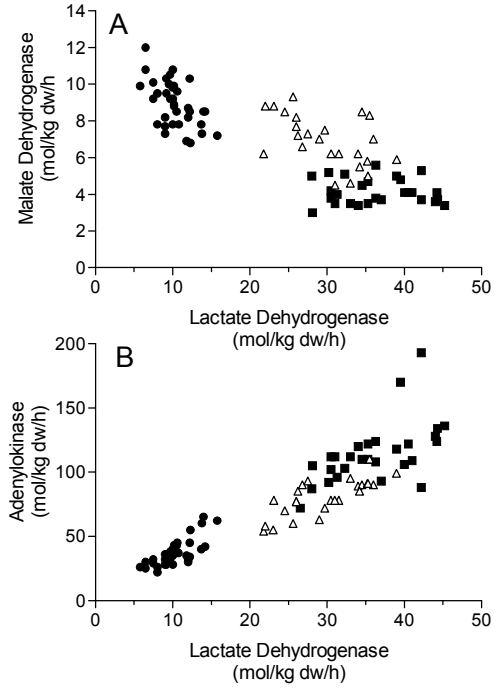

Figure 6: Relationhips between the enzymatic activities of Lactate Dehydrogenase, Malate Dehydrogenase and Adenylokinase in individual human muscle fibres. In each fibre the enzyme activities were determined and the fibre was classified as type I (filled circles), fast IIA (empty triangles) and fast IIX (squares). Note the diversity between fibre types and the large variability inside each type (from Bottinelli & Reggiani 2000).

The picture emerging from enzymatic determination in single human muscle fibres shows that the anaerobic power, i.e. the rate of ATP generation through the glycolytic pathway is 50-90% of the total (anaerobic + aerobic). The rate of aerobic-oxidative ATP production in the mitochondria is about 30% higher in slow than in fast fibres [23, 28], whereas the rate of the anaerobic-glycolytic pathway is much faster (5-10 times) in fast than in slow fibres [28]. The difference becomes relevant when compared with ATP consumption during contraction. In slow fibres the rate of ATP consumption during contraction (by myosin + ionic pumps) is estimated to be around 6 mmol/kg dry weight/s [58] and can be completely accounted for by the ATP regeneration through aerobic-oxidative processes. By contrast in fast fibres the rate of ATP consumption during maximal contractions is much higher (18-27 mmol/kg dry weight/s, [58]) and cannot be covered by the total metabolic (aerobic+anaerobic) power of the fibre. This observation helps to understand why slow, but not fast fibres, can cope with prolonged and repeated contractile activity without undergoing fatigue (see for further discussion [6, 54]).

TABLE 1: Average CSA, Po, Ao and tension cost (Po/Ao measured in pmol/mN.mm.s) in human single fibres of vastus lateralis, determined in vitro *at 20°C (data from Stienen et al 1996)*

	CSA μm^2	Po mN/mm^2	Ao mMATP/s	Po/Ao
Type I fibres	9278	114	0.10	0.87
Type IIA fibres	7922	136	0.27	1.98
Type IIX fibres	6294	171	0.41	2.39

Plasticity of human skeletal muscles

The picture of muscle fibres heterogeneity which emerges from the previous sections implies two questions which deserve answer: how inter-fibre diversity is generated and maintained and which advantage is given to muscles by the inter-fibre diversity.

Fibre composition of a given muscle can change significantly under the influence of physiological and pathological factors. This property is referred to as muscle plasticity. It appears that muscle heterogeneity and plasticity allows the muscle to respond to a wide range of mechanical demands. Factors that have been found to determine and/or regulate muscle plasticity include mechanical demand, neural activity, hormonal output and pathological conditions such as diabetes and muscular diseases [31].

Optimisation of muscle performance based on selective fibre recruitment

The diversity between muscle fibres can be best exploited if motor neurons can select which muscle fibres are convenient to activate, in order to perform a given motor task. Fast and slow, weak and strong, fatiguing and fatigue resistant fibres are available in each muscle and the most suitable in view of a given task can be recruited. According to Henneman's size principle [2, 32,], 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), and finally fast fatigue (FF) motor units are recruited only for the fastest and strongest movements (jumping). As S motor units are the least fatigable and the weakest, and FF the most fatigable and the strongest, the size principle has long been considered a way to allow a smooth accumulation of force during contraction and to optimise 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 IIB fibres, a recruitment order according to the size principle would at the same time also optimise power and efficiency.

Whether the size principle holds in all conditions, and especially in humans, is largely debated [21, 34, 41,59]. Significant modifications in the size principle have been suggested. The idea that motor units can be organised into groups recruited in order during a motor task, i.e. into task groups, has been introduced [17]. It has also been suggested that a task group does not necessarily correspond to a muscle, but can dynamically change for subtle changes in the motor task, and can even span different muscles in the same group [14, 18]. Task groups, therefore, can account for the observation that in some cases 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 holds, as within a defined task group, if not within a muscle, motor units are recruited in the order S>FR>FF. Finally, real exceptions to the size principle, not accounted for by the existence of task groups, have been shown during eccentric contractions [41].

Conclusions and Perspectives

The muscle fibres of human skeletal muscle are very heterogeneous in their functional properties and their molecular structure. This heterogeneity can be detected in all possible aspects of muscle contractile activity and is aimed to optimise the motor performance and minimise fatigue. Motor control mechanisms inside the nervous system learn how to best exploit the diversity between muscles and motor units. Inter-individual diversity in muscle performance can arise from the differences in fibre composition of muscles, the differences in motor unit composition of muscles, as well as the ability of the nervous system to organise their activation in the best way.

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