Human skeletal muscle: transition between fast and slow fibre types

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Abstract Human skeletal muscles consist of different fibre types: slow fibres (slow twitch or type I) containing the myosin heavy chain isoform (MHC)-I and fast fibres (fast twitch or type II) containing MHC-IIa (type IIA) or MHC-IIId (type IID). The following order of decreasing kinetics is known: type IID>type IIA>>type I. This order is especially based on the kinetics of stretch activation, which is the most discriminative property among fibre types. In this study we tested if hybrid fibres containing both MHC-IIa and MHC-I (type C fibres) provide a transition in kinetics between fast (type IIA) and slow fibres (type I). Our data of stretch activation kinetics suggest that type C fibres, with different ratios of MHC-IIa and MHC-I, do not provide a continuous transition. Instead, a specialized group of slow fibres, which we called “transition fibres”, seems to provide a transition. Apart of their kinetics of stretch activation, which is most close to that of type IIA, the transition fibres are characterized by large cross-sectional areas and low maximal tensions. The molecular cause for the mechanical properties of the transition fibres is unknown. It is possible that the transition fibres contain an unknown slow MHC isoform, which cannot be separated by biochemical methods. Alternatively, or in addition, isoforms of myofibrillar proteins, other than MHC, and posttranslational modifications of myofibrillar proteins could play a role regarding the characteristics of the transition fibres.

Keywords Skeletal muscle · Muscle fibre types · Myosin heavy chain · Stretch activation · Muscle mechanics · Human muscle fibres · Muscle fiber · Skeletal muscle fiber · Kinetics

Introduction

Skeletal muscles are composed of fibres with different properties to fulfill various functional needs. Contraction properties of fibres vary depending on differences in the kinetics of Ca$^{2+}$ activation, energy metabolism and isoforms of myofibrillar proteins. For classification of fibre types, morphological, metabolic and contraction properties have been used. During the last decades, the classification based on the isoforms of myosin heavy chain (MHC)
became most prevalent [20, 22]. The head portion of this protein is the essential component of the force-generating mechanism of muscles, and thus, MHC isoforms do most probably determine functional properties of the myofibrils. However, isoforms of other myofibrillar proteins, such as myosin light chains (MLC) or thin filament proteins may therefore also play a role.

Based on MHC classification three fast fibre types have been identified in adult mammalian limb muscles: IIB, IID (also called type IIX) and IIA fibres, containing the isoforms MHC-IIb, MHC-IId (x) and MHC-IIa, respectively. The slow type I fibres contain MHC-I. The group of type II fibres are often called “fast-twitch fibres” or simply “fast fibres”, whereas the type I fibres are called “slow-twitch fibres” or “slow fibres”.

Previous single fibre studies have shown that MHC isoforms correlate conspicuously with the kinetics of stretch-induced delayed force increase (stretch activation) [12, 13, 15]. This correlation most likely points to different speeds of force-generating power strokes in different MHC isoforms. The following order of decreasing kinetics has been established: MHC-IIb>MHC-IId>MHC-IIa>MHC-I.

In human, MHC-IIb is absent, and thus, the functional needs of the musculoskeletal system must be provided by only the three fibre types I, IIA and IID. Therefore, hybrid fibre types, containing more than one MHC isoform in one single fibre, could be especially important [23]. Hybrid fibre types are frequent; they are perhaps the result of different gene expression of different nuclei in the multinucleated skeletal muscle fibres. In addition to hybrid MHC isoform content, the contribution of other myofibrillar protein isoforms than MHC could play a particularly important role [4]. This issue is probably most relevant at the transition between type IIA and type I fibres because there is a large gap between the kinetics of these two fibre types. In hybrid fibres of small mammals, it was found that the kinetics of stretch activation is coarsely proportional to the ratio between MHC-IId and MHC-IIb [1, 11]. This causes a kinetic transition between the pure fibre types IID and IIB. It is unknown if a similar type of transition exists between fast and slow fibres of human muscle. In theory, the so-called type C fibres, which contain both MHC-I and MHC-IIa (type IC: [MHC-I]>[MHC-IIa], type IIC: [MHC-IIa]>[MHC-I]), could provide a continuous transition between type IIA and type I. This study was undertaken to test this assumption.

We found that the type C fibres do not provide a continuous transition. Instead, a specialized group of slow fibres seems to exist (“transition fibres”), which contribute to the transition zone by exhibiting relatively fast kinetics and—interestingly—they showed conspicuously large cross-sectional areas and low tensions.

Materials and methods

Muscle preparations

Muscle samples of a few millimetres in size were obtained from the following human muscles: sternocleidomastoidus, vastus lateralis, tibialis posterior, teres major, gluteus medius, erector spinae and finally deltoideus. The samples originated from patients (16–75 years) undergoing surgery. The patients were informed and asked for their written consent and permission. Immediately after excision the samples were incubated in a cold skinning solution. After washing, muscle samples were split into strips of approximatively 1–2-mm diameter.

The following five solutions (0–5°C) were used for sequential incubations: (1) skinning solution meaning 132 mM Na propionate, 5 mM ethylene glycol tetraacetic acid (EGTA), 7 mM Na$_2$H$_2$ATP, 2 mM MgCl$_2$, 10 mM 3-(N-morpholino)propanesulfonic acid, 2 mM dithioerythritol (DTE), 30 mM 2,3-butadiene monoxime; pH adjusted to 6.9 with 132 mM KOH; (2) skinning solution in which Na propionate was replaced by K propionate; (3) solution 2 with 10% (v/v) glycerol; (4) solution 2 with 25% (v/v) glycerol; and (5) relaxation solution (pH 6.9) with 50% (v/v) glycerol. The preparations were stored in solution 5 at about −25°C. Before the experiments, single fibres were dissected in this solution and glued to the tip of two needles of the apparatus.

Mechanical measurements

The experimental apparatus and the method for mechanical measurements have been described previously [9]: The attachment points for the skeletal muscle fibres (active length, about 2 mm; mean diameter about 30–120 μm) on the mechanical apparatus were two vertical epoxy carbon fibre needles of about 100-μm tip diameter. The needles were connected to the rest of the apparatus by silicon plates from force transducer elements (AE 801, SensoNor, Norway). One element, the force sensor, was connected mechanically to a micrometer screw and electrically to a force bridge amplifier. The other element was a dummy, glued on the lever arm of a stepping motor. Rapid changes of the fibre length (≤1 ms) were achieved by a feedback-controlled stepping motor based on a Ling vibrator. The ability to make rapid solution changes (about 0.2 s) was provided by a cuvette transporting system. Laser diffractometry (He–Ne laser, 632.8 nm, 4 mW) was used for measuring sarcomere length.

After attachment of the skinned preparation with tissue adhesive (Vetsel, Braun, Melsungen, Germany), the fibre ends were fixed by superfusion for 2–3 s with a fine, rapidly flowing, downward-directed stream of stained
glutaraldehyde solution (8% glutaraldehyde, 5% toluidine blue, fixative). For this purpose the preparation was bathed in a rigor solution with low ionic strength (1 mM MgCl2, 10 mM imidazole, 2.5 mM EGTA, 134 mM potassium propionate; pH 6.9) with a lower specific mass than the fixative for less than 30 s. This procedure created a sharp boundary between the functional part of the preparation and the fixed ends. This method improves considerably the maintenance of the sarcomere homogeneity and the stability of the mechanical properties during prolonged activations [14].

The solutions used during the mechanical measurements had an ionic strength of 0.25 M and contained 60 mM HEPES, 8 mM Na2H2ATP, 10 mM sodium creatine phosphate, 1 mM DTE, 40 g/l dextran T-500, 30 U/ml creatine phosphokinase and 1 mM free Mg2+. In addition, the relaxing and activation solutions contained 50 mM EGTA. The pCa was >8 in the relaxation solution and 5.4 in the activating solution by addition of CaCO3. The preactivating solution (low Ca2+-buffering capacity) contained 50 hexamethylenediamine-N,N,N′,N′-tetraacetic acid and had a pCa of about 7. pH was adjusted to 7.10 at 22°C in all solutions. The pCa and the free [Mg2+] of the solutions were determined with ion-selective electrodes.

Prior to the experiment, the length of the preparations was adjusted to exactly the slack position in relaxing solution and both fibre dimensions and sarcomere length were recorded. The cross-sectional area of the individual fibres was calculated using their largest and smallest diameter and assuming an elliptic shape. After transferring the fibre to the preactivating solution (ca. 0.5 min) and subsequently to the activating solution a steady, maximal force was reached. For calculating the maximal (isometric) tension, the maximal force was divided by the cross-sectional area of the individual fibre. A number of quick stretches (≤1 ms; 0.2–0.4% of fibre length) were applied to induce force responses including stretch activation. Time parameters (t5, t5; see “Results”) were measured on single force responses and averaged for each fibre. The experiments were carried out at 22°C.

Biochemical analysis

After completion of mechanical measurements, the muscle preparations were removed from the apparatus for biochemical analysis. The preparations were dissolved in sodium dodecyl sulfate (SDS) lysis buffer (62.5 mM Tris–HCl, pH 6.8, 10% glycerol v/v, 2.3% SDS w/v, 5% 2-mercaptoethanol v/v) and heated at 65°C for 15 min. Subsequently, a part of this extract (ca. 0.1 μg protein) was applied to a SDS polyacrylamide gel system (4% polyacrylamide in stacking gel and 8% in separation gel). For electrophoresis, a SE 600 chamber (Hoefer, San Francisco, California, USA) with 16-cm long glass plates was used [12]. A constant voltage of 180 V was applied for 25 h. During the experiment the electrophoresis chamber was cooled with running water. After electrophoresis, gels were silver stained and the relative amount of MHC isoforms was estimated by densitometry.

Statistical and data analysis

Data were analyzed using Microsoft Excel 2003® for Windows® and Statistical Package for the Social Sciences (SPSS®; 18). Results are presented as mean ± standard deviation. The data were analyzed for normal distribution by the Kolmogorov–Smirnov test. Differences between groups were tested by one-way ANOVA, unpaired t tests or, in case of no normal distribution, by the Mann–Whitney U test.

Results

Myosin heavy chain analysis

A total number of 225 type I fibres and 104 type IIA fibres were found. Fibres containing MHC-IId were discarded because they were not in the focus of the study. A total of 41 fibres were found which contained both MHC-I and MHC-IIa (type C fibres). These fibres can be divided in type IC containing more MHC-I than MHC-IIa (n=23) and type ICC containing more MHC-IIa than MHC-I (n=18). The ratio [MHC-I]/([MHC-I]+[MHC-IIa]) of individual fibres is visible in Fig. 1c. It ranged between about 5% and 95%. Interestingly, fibres containing 40–75% of MHC-I were rare.

Stretch activation experiments

After maximal isometric activation, stepwise stretches were applied. Quick stretches caused an immediate force increase. When the new fibre length was reached, force decreased and increased again (usually designated as “delayed force increase” or “stretch activation”; Fig. 1a). For evaluation, the time from the beginning of the stretch to the lowest force value before the onset of the delayed force increase (t2) and to the peak value of the delayed force increase (t3) was measured.

As shown in Fig. 1b, the t2 values (mean ± SD) of fibre types decrease in the order I>IC>>IIC>IIA. There is no overlap between the t2 values of pure fibre types I and IIA. This is indicated by the standard deviations shown in this figure (and by the individual data points presented in Fig. 1c). The t2 values showed a pattern similar to that of the t3 values. Figure 1c shows the t3 values of type C and
flanking pure fibre types in relation to their MHC isoform content. The distribution suggests that in type C fibres the kinetics is hardly proportionally related to the relative MHC isoform content.

Figure 2 shows the correlation between individual \( t_3 \) and \( t_2 \) values. The diagram demonstrates that two groups with different kinetics can be separated: a “slow group” and a “fast group”. The slow group comprises the type I and type IC fibres whereas the fast group comprises type IIA and type IIC fibres.

To analyze the transition zone, a group of slow fibres with kinetics most closely related to those of fast fibres was selected. Inclusion criteria for these “transition fibres” were \( t_2 \) values between 160 and 275 ms and \( t_3 \) values between 700 and 1,400 ms (see circle in Fig. 2). These transition fibres contained 14 type I (100% MHC-I) fibres and 6 type IC (58–89% MHC-I) fibres. The transition fibres originated from the following muscles: vastus lateralis \((n=7)\), tibialis posterior \((n=6)\), erector spinae \((n=5)\), deltoideus \((n=1)\) and sternocleidomastoideus \((n=1)\).

The transition fibres showed further common properties which are displayed in Fig. 3 in comparison with other fibre types. The \( t_3 \) values of the transition fibres \((1,109±174 \text{ ms})\) ranged between type IC \((2,088±729 \text{ ms}; \text{transition fibres excluded})\) and IIC fibres \((330±88 \text{ ms})\).

Moreover, the transition fibres revealed low maximal force \((0.45±0.016 \text{ mN})\), which was similarly low to that of type IC fibres \((0.39±0.20 \text{ mN})\). The next higher force was observed in type I fibres \((0.51±0.26 \text{ mN})\) followed by type IIC \((0.69±0.37 \text{ mN})\) and finally type IIA \((0.80±0.34 \text{ mN})\).

Interestingly, the transition fibres had conspicuously large cross-sectional areas \((4,635±1,544 \mu \text{m}^2)\). On average, the area was about 2.3 times larger than the area of type IC \((1,922±917 \mu \text{m}^2)\) and IIC \((2,124±1,134 \mu \text{m}^2)\) fibres and about 1.7 times larger than the area of type I \((2,854±1,667 \mu \text{m}^2)\) and type IC fibres \((2,500±1,572 \mu \text{m}^2)\) fibres.

The transition fibres exhibited markedly low maximal tensions \((107±53 \text{ mN mm}^{-2})\). Tension was about 3.6 times lower in transition fibres than in type IIC \((377±181 \text{ mN mm}^{-2})\) and IIA \((402±209 \text{ mN mm}^{-2})\) fibres and about 2.1 times lower than in type I \((224±136 \text{ mN mm}^{-2})\) and IC fibres \((225±117 \text{ mN mm}^{-2})\).

In summary, with regard to \( t_3 \) and maximal force, the transition fibres were in between type IC and IIC fibres. However, regarding cross-sectional area, the transition fibres clearly surpassed type C (and type I and IIA) fibres. Furthermore, regarding maximal tension, the transition fibres were markedly inferior to type C (and type I and IIA) fibres.
Discussion

This study analyzed functional properties of human skeletal muscle fibres in order to achieve an understanding of the transition zone between slow and fast fibres. The kinetics of stretch activation (in particular $t_3$) was considered as the most significant property because it is known to be most discriminative among fibre types. The results of this research demonstrate that fibres classified as type C, i.e. containing different MHC-I/MHC-IIa ratios, do not provide a continuous transition between fast and slow fibres. Instead, a specialized group of slow fibres, the so-called transition fibres, seems to fulfil this function.

In the present study, a relatively large number of type C fibres were found so that detailed statements about their kinetics are possible. The few type C fibres found in previous studies exhibited $t_3$ values with a mean located almost exactly between corresponding pure fibres [13], which was suggestive of type C fibres providing a transition between fast and slow fibres. Instead, a specialized group of slow fibres, the so-called transition fibres, seems to fulfil this function.

Since the transition between fast and slow fibres is obviously not (only) provided by type C fibres, this transition might be provided by specialized slow fibres, i.e. the “transition fibres”. If these transition fibres are defined as a separate unit, the following continuous sequence of kinetics can be stated (from fast to slow): type IIA$>$type IIC$>$transition fibres$>$type IC$>$type I (Fig. 3). The group of transition fibres is not only characterized by kinetics in between fast and slow fibres but also by further common properties (Fig. 3). Therefore, the transition fibres could represent an own entity of fibres. The most striking property of this transition group was the conspicuously large cross-sectional area, which was the largest among all fibre types. Due to their large size, one would expect high forces from them. However, these fibres were conspicuously weak. The weakness is either due to a low portion of myofibrils within the fibres or to low tension of the myofibrils. If the portion of myofibrils is low, it is possible that the remaining volume of the fibres is predominately filled with mitochondria. This would indicate a continuous mechanical activity of these fibres.

Fig. 2 Correlation between the time parameters $t_3$ and $t_2$ of stretch activation. The diagram shows the values of individual fibres belonging to type I (empty rhombus), type IC (filled square), type IIC (filled triangle) and type IIA (plus). The “transition fibres” are wrapped by the circle.
The fact that the transition fibres exhibited large cross-sectional areas and low maximal tensions must depend on the physiological needs of these fibres. In this context, their abundance in specific muscles and their time pattern of neuronal recruitment plays a role. Further studies are required to elucidate these functional aspects. At present it can simply be noted that these specialized transition fibres with common properties do exist. Transition fibres are known also in other muscle systems. Moreover, the rabbit masseter muscle contains many fibres exhibiting the so-called MHC alpha isoform (which probably is identical with the alpha-cardiac MHC). These alpha fibres close the kinetic gap between fast and slow fibres in this specific muscle of rabbits [3]. In the present study, MHC alpha was not found.

The observation that transition fibres exist raises the question about the underlying cause for their specific properties. At present this question cannot be answered. However, various possibilities (which are perhaps additive) can be addressed:

1. It seems possible that the electrophoretic MHC-I band did not represent a single isoform, but two or even more MHC isoforms with different kinetics. In most studies the electrophoretic analysis failed to identify any diversity of slow MHC isoforms. Perhaps, the differences in the amino acid sequence (total number of amino acids, 1,940 [17]) of these isoforms were too small to be detectable by available biochemical methods. However, in some other studies, a separation of MHC-I into two bands was observed [8, 10]. Therefore, it seems possible that the characteristics of the transition fibres, identified in this study as a part of slow fibres, are due to a specific MHC isoform not yet identified by biochemical methods.

2. Posttranslational modifications of MHC could cause protein variations with altered functions [16, 19, 21]. Among these, the possibility of different glycation and/or oxidation status of MHC could be important. Indeed, glycation of myosin is known to influence filament sliding in in vitro motility assays [21].

3. Isoforms of other myofibrillar proteins such as MLC and thin filament proteins could influence myofibrillar kinetics [5, 6]. Thus, it is known that the presence of slow alkali MLC isoforms in type IIA fibres of rat and rabbit is associated with decelerated stretch activation kinetics [9]. Therefore, it might well be that the presence of fast alkali MLC isoforms in slow fibres (such as in the here identified transition fibres) may accelerate the kinetics. The MLC complement was not investigated in fibres of this study, and therefore, this possibility cannot be verified. Similar possibilities exist for the regulatory MLC isoforms. Here, in addition, a different phosphorylation status (known to influence mechanical properties [7]) could play a role. Also, isoforms of the regulatory protein troponin C (TnC) could influence the kinetics. This possibility can be deduced from experiments in which skinned fibres of rat

Fig. 3 Properties of the “transition fibres” in comparison with other fibre types. The diagram shows means ± SD values of \(t_3\), maximal force, cross-sectional area and maximal tension of type I, type IC, “transition fibres” (Tr.F.), type IIC and type IIA fibres. The number of fibres, \(n\), is given under parenthesis. The comparison between the five groups revealed highly significant differences for all properties (ANOVA, \(p \leq 0.001\)). The symbols near the columns represent levels of significance from comparisons with the “transition fibres” using \(t\) tests (*** for \(p \leq 0.001\), ** for \(p \leq 0.01\)) or Mann-Whitney \(U\) tests (+++ for \(p < 0.001\)).

The fact that the transition fibres exhibited large cross-sectional areas and low maximal tensions must depend on the physiological needs of these fibres. In this context, their abundance in specific muscles and their time pattern of neuronal recruitment plays a role. Further
diaphragm were activated by Sr\(^{2+}\) (instead of Ca\(^{2+}\)) [18]. In these experiments it was observed that TnC isoforms determine the relationship between force and [Sr\(^{2+}\)].

The present study gives rise to reconsider the meaningfulness of fibre type classification. The common use of MHC isoforms for categorizing fibre types is definitely an important approach for classification. However, increasing knowledge suggests that variations in MHC isoforms are not sufficient for fulfilling all functional needs of fibres; isoforms of other myofibrillar proteins and probably also posttranslational modifications of these proteins may play a role. Furthermore, protein modifications may not be constant but adapted in a relatively short time. Therefore, fibre type classification is difficult. Finally, it can be stated that any type of classification may not be considered as a realistic image of nature but rather as a makeshift helpful in the daily routine.

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