# Force–velocity properties of human skeletal muscle fibres: myosin heavy chain isoform and temperature dependence

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1. A large population ($n = 151$) of human skinned skeletal muscle fibres has been studied. Force–velocity curves of sixty-seven fibres were obtained by load-clamp manoeuvres at 12 °C. In each fibre maximum shortening velocity ($V_{max}$), maximum power output ($\dot{W}_{max}$), optimal velocity (velocity at which $\dot{W}_{max}$ is developed, $V_{opt}$), optimal force (force at which $\dot{W}_{max}$ is developed, $P_{opt}$), specific tension ($P_o$/CSA, isometric tension/cross-sectional area) were assessed. Unloaded shortening velocity ($V_o$) was also determined at 12 °C in a different group ($n = 57$) of fibres by slack-test procedure.

2. All fibres used for mechanical experiments were characterized on the basis of the myosin heavy chain (MHC) isoform composition by sodium dodecyl sulphate (SDS)–polyacrylamide gel electrophoresis and divided into five types: type I (or slow), types II A and II B (or fast), and types I–II A and II A–II B (or mixed types).

3. $V_{max}$, $\dot{W}_{max}$, $V_{opt}$, $P_{opt}$, $V_{opt}/V_{max}$ ratio, $P_o$/CSA and $V_o$ were found to depend on MHC isoform composition. All parameters were significantly lower in type I than in the fast (type II A and II B) fibres. Among fast fibres, $V_{max}$, $\dot{W}_{max}$, $V_{opt}$ and $V_o$ were significantly lower in type II A than in II B fibres, whereas $P_{opt}$, $P_o$/CSA and $V_{opt}/V_{max}$ were similar.

4. The temperature dependence of $V_o$ and $P_o$/CSA was assessed in a group of twenty-one fibres in the range 12–22 °C. In a set of six fibres temperature dependence of $V_{max}$ was also studied. The $Q_{10}$ (5·88) and activation energy $E$ (125 kJ mol$^{-1}$) values for maximum shortening velocity calculated from Arrhenius plots pointed to a very high temperature sensitivity. $P_o$/CSA was very temperature dependent in the 12–17 °C range, but less dependent between 17 and 22 °C.

The influence of myosin isoform composition on the contractile properties of isolated human skeletal muscle fibres has been studied only very recently. Unloaded shortening velocity ($V_o$) determined by the slack-test technique has been shown to be mostly, even though not solely, determined by the myosin heavy chain (MHC) isoform composition of the fibres (Larsson & Moss, 1993). In the latter study, mean $V_o$ values at 15 °C were found to vary between 0·31 L s$^{-1}$ (fibre lengths per second) for fibres containing MHC I isoform and 3·04 L s$^{-1}$ for fibres containing MHC II B, with intermediate values for MHC II A-containing fibres. Isometric tension ($P_o$) has not been unequivocally related to MHC composition of the fibres.

In human fibres, besides $V_o$, other force–velocity properties such as maximum power output ($\dot{W}_{max}$), and optimal velocity (velocity at which $\dot{W}_{max}$ is developed or $V_{opt}$) have not so far been studied, and their relationship with myosin isoforms is unknown. This is a relevant issue for at least two reasons. Muscles contracting *in vivo* in the body do not shorten at their maximum shortening velocity, but at the velocity at which maximum power output is developed (Rome *et al.* 1988). Therefore, maximum shortening velocity, which is the most extensively studied among the contractile properties *in vitro* as it gives relevant information on the kinetics of actomyosin interaction, is of limited importance *in vivo*. Moreover, $\dot{W}_{max}$ can be determined more reliably than both $V_o$ (maximum shortening velocity determined by slack test) and $V_{max}$ (maximum shortening velocity determined by extrapolation of force–velocity curves; Brooks, Faulkner & McCubbrey, 1990; Seow & Ford, 1991). $V_o$ determination might be impaired by a long lasting component of series elasticity (Seow & Ford, 1992). $V_{max}$, being obtained by extrapolation to zero load of force–velocity curves, could be significantly affected by even small errors or deviations from the hyperbolic shape in the low-load region of the curve (Julian, Rome, Stephenson & Striz, 1986; Bottinelli, Schiaffino & Reggiani, 1991). $\dot{W}_{max}$, which is derived from the intermediate portion of the force–velocity curve where velocity changes less steeply with load, is mostly free from uncertainties that might affect not only $V_o$, but also $V_{max}$ (Brooks *et al.* 1990; Seow & Ford, 1991; Moss, Diffee & Greaser, 1995).

In amphibians and small mammals, temperature dependence of mechanical and biochemical properties of skeletal muscle has been studied in great detail (Edman, 1979; Ranatunga,

1982, 1984; Stein, Gordon & Shriver, 1982; Siemankowski, Wiseman & Howard, 1985; de Tombe & Ter Keurs, 1990; Pate, Wilson, Bhimani & Cooke, 1994). On the contrary, in humans, apart from a very recent study in which the temperature dependence of isometric tension and ATPase activity have been explored over a wide temperature range (12–30 °C, Stienen, Kiers, Bottinelli & Reggiani, 1996), all studies on single fibres have been performed at temperatures (12–15 °C) far below the muscle temperature in the body.

This study aims: (1) to assess whether force–velocity properties of single human skeletal fibres, some of which (i.e. $W_{max}$ and $V_{opt}$) are of critical importance *in vivo*, are related to myosin heavy chain isoform composition; and (2) to assess the temperature dependence of maximum shortening velocity and isometric force in human skeletal fibres.

To address the first aim, force–velocity relationships of a large population ($n = 67$) of human skinned fibres from the vastus lateralis muscles were determined at 12 °C. Another group of fibres ($n = 57$) was subjected to slack-test manoeuvres for $V_0$ determination. $V_0$, $V_{max}$, $\dot{W}_{max}$, $V_{opt}$, $P_0$ and other force–velocity properties were tested for a possible relationship with the MHC isoform composition of the fibres determined by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE). MHC isoform composition was found to affect not only $V_0$ and $V_{max}$, but also $\dot{W}_{max}$, $V_{opt}$, $P_0$ and the shape of the force–velocity relationship.

To assess the temperature dependence of maximum shortening velocity and isometric force, $V_0$ and $P_0$ of a separate group of fibres ($n = 21$) were determined at 12 °C (control temperature) and either at 17 or 22 °C (test temperatures). $Q_{10}$ values (temperature coefficient over the 12–22 °C temperature range) and activation energy ($E$) were calculated using Arrhenius plots. Due to technical difficulties, the temperature dependence of $V_{max}$ was studied only in a smaller group of fibres ($n = 6$) and in the narrower temperature range of 12–17 °C.

A preliminary report of this study has been published in abstract form (Bottinelli, Canepari, Pellegrino, Zanardi & Reggiani, 1996).

## METHODS

### Muscle biopsies and dissection

Biopsy samples were taken from the vastus lateralis muscles of six male subjects, aged 30–50 years, who were healthy and without any previous history of muscular or neuromuscular disease. Needle biopsy samples (50–100 mg) were taken from four subjects under local anaesthesia with Xylocaine (Stienen *et al.* 1996), from the distal portion of the vastus lateralis muscle. Two more biopsy samples (100–200 mg) were taken during orthopaedic surgery. The study was approved by the Institute of Human Physiology ethical commitee and written consent was given. Fresh biopsies were immersed in ice-cold skinning solution and divided into small bundles of about fifty fibres each, which were then stored in skinning solution with 50% added glycerol at −20 °C for up to 1 month. On the day of the experiment, a bundle was transferred in skinning solution at 10–12 °C and, under a stereomicroscope (Wild × 10–60), single fibre segments 2–4 mm long were dissected out by pulling lengthwise. At the end of the dissection, fibres were bathed for 1 h in a new skinning solution containing 1% Triton X-100 (Sigma) to ensure removal of the sarcolemma and sarcoplasmic reticulum. Light aluminum clips were used to attach the fibre segments to the beams of the force transducer and isotonic lever in the experimental set-up.

### Experimental set-up

The experimental set-up is similar to that previously described in detail (Bottinelli *et al.* 1991; Bottinelli, Betto, Schiaffino & Reggiani, 1994*a*). Three muscle chambers were milled in an aluminum plate in which a water–glycerol solution was circulated for temperature control by an Endocal Neslab thermostat. One chamber (12 mm long, 7 mm wide and 1 mm deep) containing relaxing solution was used to mount the fibre. The two other chambers were smaller (6 mm long, 3 mm wide and 1 mm deep) and contained pre-activating and activating solutions. Coverslips were suspended 2 mm above the small chambers by means of a long movable arm. The arm could rotate to cover and uncover the chambers. Drops (70 μl) of either preactivating or activating solution filled not only the small chambers, but also the gap between the small chambers below and the coverslips above. The beam of the force transducer (AE 801 Aksjeselskapet Mikro-elektronikk, Horten, Norway; resonance frequency, 2 kHz) and of the electromagnetic puller (model 101 vibrator, Ling Dynamic System, Royston, Herts, UK) entered horizontally the drops between the chambers and the coverslips. This allowed quick transfer of the fibre from relaxing to pre-activating and from pre-activating to activating solutions simply by horizontal shift of the aluminum plate. The electromagnetic puller, provided with an inductance position transducer and driven by a feed-back circuit, could either keep the length of the fibre segment constant to elicit isometric contractions, or keep the load applied to the specimen constant to elicit isotonic shortening against different loads, or impose on the specimen quick releases of pre-set amplitude completed in 2 ms. A stereomicroscope was fitted over the apparatus to view the fibre during the mounting procedure and the experiment. The set-up was placed on the stage of an inverted microscope (Axiovert 10, Zeiss, Germany). As the floors of the muscle chambers were made by coverslips, specimens could be viewed at ×320 magnification through the eyepieces of the microscope. A video camera fitted to the camera tube of the microscope (MICAM HRS, System Sud, Les Ulis, France) and connected through an A/D converter (Cyclope, System Sud, Les Ulis, France) to a computer (Olivetti M24) allowed viewing on a TV screen at approximately ×1000 magnification, and storage of digitized images of the specimen during the experiment (Fig. 1).

### Solutions

Skinning, relaxing, pre-activating and activating solutions were prepared according to Bottinelli *et al.* (1994*a*) with some modifications. The EGTA concentration in the pre-activating ($Ca^{2+}$ free) solution was decreased from 5·0 to 0·5 mM to induce faster $Ca^{2+}$ diffusion inside specimens and therefore quicker activation (Larsson & Moss, 1993). Free ionic concentration and ionic strength of all solutions were calculated using a computer program designed by Fabiato (Fabiato, 1988). In relaxing and activating solutions ionic strength was 0·20 M. In relaxing, pre-activating and activating solutions imidazole (20 mM) was the pH buffer and $Cl^-$

(120 mM) was the main anion. Relaxing, pre-activating and activating solutions to be used at 17 and 22 °C were slightly modified to keep values of free concentration of all ions, ionic strength and pH similar to those at 12 °C. The pH of the solutions was adjusted to 7·0 at the temperature at which solutions were used. The amount of KOH added to buffer was considered in making solutions in order to maintain the free potassium concentration constant. Pre-activating and activating solutions contained a rephosphorylating system based on creatine phosphokinase (Boehringer Mannheim, Germany; 300 U $ml^{-1}$) and creatine phosphate (Boehringer Mannheim; 25 mM; Bottinelli *et al.* 1991).

### Experimental plan

A total of 151 fibres were successfully analysed in mechanical experiments. Load-clamp manoeuvres were performed on 67 fibres to obtain force–velocity relationships. All parameters that characterize the force–velocity curve were determined. Maximum shortening velocity ($V_{max}$) was obtained by extrapolation of the curve to zero load. Fifty-seven fibres were subjected to slack-test manoeuvres to determine directly maximum shortening velocity ($V_0$). The temperature dependence of maximum shortening velocity and isometric force was assessed in twenty-one fibres by a slack test in the temperature range 12–22 °C and in six fibres by load clamps and force–velocity determination at 12 and 17 °C. At the end of all the mechanical experiments, fibres were removed from the apparatus and thereafter characterized on the basis of MHC isoform composition by SDS–PAGE.

### Experimental procedure

In all the mechanical experiments the fibre segment was mounted in a chamber containing relaxing solution; sarcomere length (SL) and fibre diameter were measured at three different locations along the length of the specimen at ×320 magnification using the eyepieces of the inverted microscope and SL, determined by counting striations in segments of known length, was set at 2·5 μm; segment length was measured using the stereomicroscope

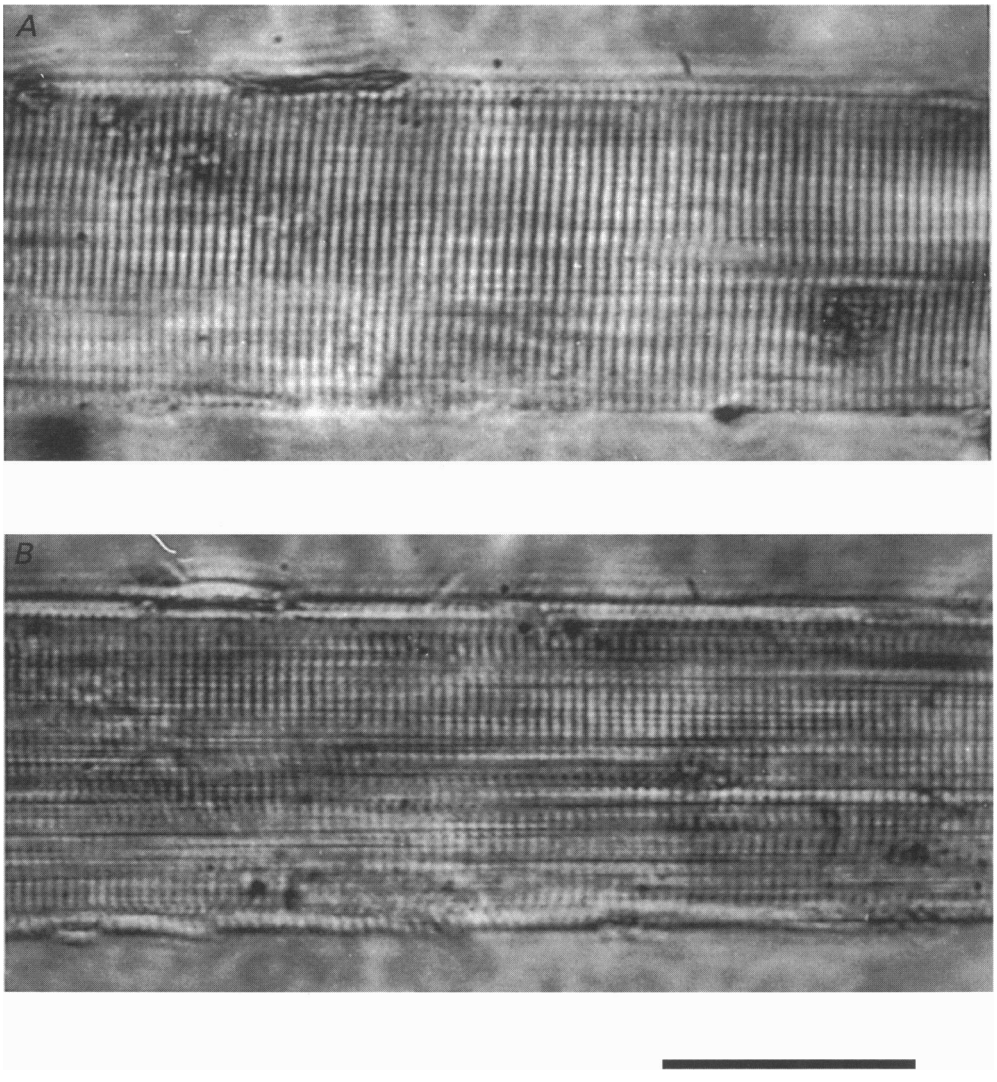


**Figure 1. Photomicrographs of a human skinned skeletal muscle fibre in relaxing (*A*) and activating (*B*) solutions**

Photomicrograph in panel *B* was taken at the end of 3 min maximal activation for force–velocity determination. Scale bar, 50 μm.

fitted over the apparatus at ×40 magnification. Cross-sectional area (CSA) of the specimen was determined assuming a circular shape from the mean of the three diameters measured at ×320 magnification, without correction for swelling. Fibres were first transferred to a pre-activating solution for at least 2 min and then maximally activated (pCa, 4·45). To determine force–velocity relationships, fibres were subjected to fifteen to twenty-five load clamps during a single activation lasting 1·5–3 min. Examples of load clamps are shown in the inset of Fig. 2*A*. To determine $V_0$, slack-test manoeuvres were employed. Each fibre was subjected to five to six short (40–50 s) activations. During each activation a quick release (completed in 2 ms) of amplitude between 5 and 12% of muscle length was applied to the fibre. In all cases, such releases made the fibres go slack before redeveloping force at the final length. To study temperature dependence of maximum shortening velocity and isometric force, fibres were subjected either to two sets of four to five quick releases or to two sets of load clamps at two temperatures. Slack tests were performed at 12 °C (control temperature) and either at 17 or at 22 °C (test temperatures). Load clamps were performed at 12 and 17 °C. Slack test and force–velocity determinations at the control temperature preceded the determinations at test temperatures in half the fibres and followed them in the other half. At the end of the mechanical experiment all fibres were immersed in 20 $\mu$l of sample buffer (Laemmli, 1970), stored at −20 °C, and then characterized by SDS–PAGE.

**Data recording and analysis**

The force and length signals were fed to a storage oscilloscope (model 5113, Tektronix, Beaverton, OR, USA), to a chart recorder (Graphtec WR3701, Japan), and to a digital oscilloscope (Nicolet 4094 C, Madison, WI, USA). Analyses were carried out on the disk recordings of the digital oscilloscope.

To build force–velocity relationships, shortening velocity was measured from load-clamp manoeuvres by linear interpolation in the interval 30–40 ms after the beginning of shortening and expressed in fibre segment length per second (L s$^{-1}$). Load was expressed as $P/P_0$, where $P$ is force during load clamping and $P_0$ is the isometric force developed just prior to load clamping. Hill's hyperbolic eqn (1) (where $P$ is load at velocity $V$, $P_0^*/P_0$ is the intercept with the force axis, and $a/P_0$ and $b$ are constants) was fitted to all experimental data points using a computer program designed according to the minimization procedure described by Wohlfart & Edman, 1994.

$$(P/P_0 + a/P_0)(V + b) = (P_0^*/P_0 + a/P_0)\, b. \quad (1)$$

From the computed force–velocity curve, maximum shortening velocity (intercept with the velocity axis or $V_{max}$), curvature of the relationship ($a/P_0$), and $P_0^*/P_0$ could be obtained. Using the parameters of Hill's equation maximum power output ($\dot{W}_{max}$), and velocity ($V_{opt}$) and force ($P_{opt}$) at which $\dot{W}_{max}$ was developed could be calculated. $\dot{W}_{max}$ was expressed in two ways: (1) relative to $P_0$

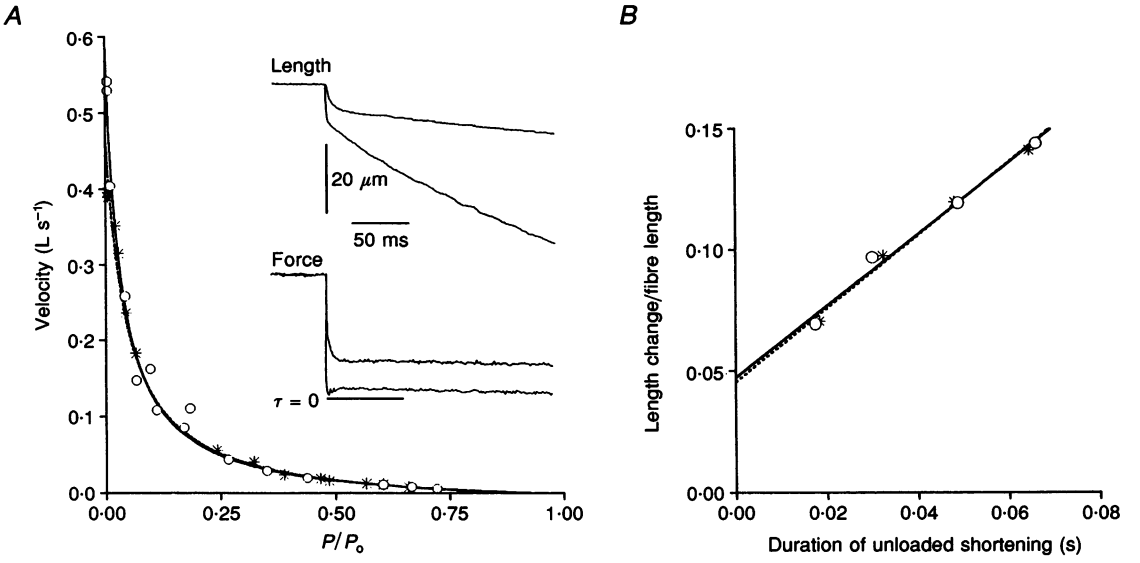


**Figure 2. Examples of force–velocity curves from load-clamp manoeuvres and of plots of slack-test data**

*A*, two force–velocity curves obtained from the same fibre segment (type II A) in two subsequent activations (○, first activation and continuous line; ✳, second activation and dashed line). Load is expressed relative to isometric force ($P_0$) and velocity in fibre length per second (L s$^{-1}$). Temperature, 12 °C; first activation: $V_{max}$, 0·583 L s$^{-1}$; $a/P_0$, 0·032; second activation: $V_{max}$, 0·483 L s$^{-1}$; $a/P_0$, 0·044. In the inset, examples of two load-clamp manoeuvres. *B*, the time (s) taken to take up the slack is plotted *vs.* the amplitude of the release (in fibre length) for two sets of slack tests obtained from the same fibre segment (type II B). Four releases of different amplitude were repeated twice in the same order in a total of eight activations to verify reliability of specimen and repeatibility of measurements. The slope of each linear regression is $V_0$ expressed in length per second. The intercept with the $y$-axis is series compliance expressed in fibre length. The slack-test data points and the corresponding linear regression lines obtained from the two sets (○, first set and continuous line; ✳, second set and dashed line) almost superimpose. $V_0$ for the first set = 1·485 L s$^{-1}$; $V_0$ for the second set = 1·520 L s$^{-1}$.

(in $P_o$ L s$^{-1}$) to compare the $\dot{W}_{max}$ of different fibre types regardless of differences in their specific tension; and (2) in absolute values (in watts per litre, W l$^{-1}$) to estimate the actual difference in the capacity to perform mechanical work of the different fibre types.

$V_o$ was determined from the slope of the linear regression between the time needed to take up the slack and the amount of the length change applied (Fig. 2*B*) and expressed in fibre segment length per second (L s$^{-1}$) (Edman, 1979). The intercept of the regression line with the length axis was the series compliance.

To determine $Q_{10}$ and activation energy ($E$) of $V_o$ the Arrhenius equation in the following form was used:

$$\log k = -\frac{E}{2{\cdot}303RT} + \text{constant}, \quad (2)$$

where $k$ is the rate parameter; $R$, the gas constant; $T$, absolute temperature; and $E$, activation energy. The logarithmic values of the ratios between the parameter at the test temperature and the control temperature were plotted *versus* the reciprocal of absolute temperature (Fig. 8*B*). From the slope of the linear regression line $E$ was calculated ($E$ = slope × 2·303$R$) and from $E$, $Q_{10}$ was determined according to:

$$\log Q_{10} = E/R(1/T_1 - 1/(T_1 + 10)), \quad (3)$$

where $T_1$ is the lower temperature used (12 °C) expressed as absolute temperature. $Q_{10}$ for $V_{max}$ was not calculated as only two temperatures were studied (12 and 17 °C). $Q_{10}$ for $P_o$ was not calculated either as $P_o$ is not a rate parameter and therefore $Q_{10}$ values should not strictly apply to it (Bennett, 1984), but also as the relationship between $P_o$ and temperature was not linear.

### Quality controls

In skinned fibres, force and especially velocity measurements can be made less reliable by the sarcomere pattern becoming disordered during prolonged and repeated maximal activations. To check the reliability of the specimen the following quality control procedures were employed. (1) Several fibres were subjected to two maximal activations for force–velocity determination. Figure 2*A* shows that the force–velocity curve obtained from the first activation was almost superimposed by the force–velocity curve obtained from the second activation. (2) Several fibres were subjected to two identical sets of four quick releases for $V_o$ determination in a total of eight activations. Figure 2*B* shows that the linear regression of the first set of releases was almost superimposed on the linear regression of the second set of identical releases. (3) The experimental procedure employed for force–velocity determination was such that it was possible to repeat several load clamps at the same load at the beginning and at the end of the long activation in all fibres. Velocities determined from the same load clamps at the beginning and at the end of activation were very similar. (4) The highly magnified image of the fibre was projected onto a TV screen throughout the experiment. The striated pattern was well preserved after repeated activations at 12 °C (Fig. 1). At 22 °C striations were still visible although some skewing and disarray of the myofibrils was observed. (5) Fibres whose force decreased by more than 10% during activation or between the first and the last activation were discarded.

### Statistical analysis

Data were expressed as means ± S.D. Statistical significance of the differences between means was assessed by variance analysis followed by the Student–Newman–Keuls test. A probability of less than 5% ($P \leq 0{\cdot}05$) was considered to be statistically significant. The statistical package, Primer in Biostatistics (by S. A. Glantz, released by McGraw-Hill), was used. Linear regression analysis was performed using a computer program (Prism, GraphPad software, San Diego, CA, USA). Slopes were considered significantly different from zero when the $P$ value was $\leq 0{\cdot}05$.

### SDS–PAGE

Myosin heavy chain (MHC) isoform composition was determined by means of polyacrylamide gel electrophoresis after denaturation in sodium dodecyl sulphate (SDS–PAGE) by a method derived with some modifications from Danieli-Betto, Zerbato & Betto (1986) and Stienen *et al.* (1996). In short, after each successful series of measurements, the fibre segment was placed in a small test tube filled with 20 μl of sample buffer (Laemmli, 1970). A small fraction of the solution (typically 5 μl) containing approximately 400–1000 ng proteins was loaded onto gels. Slabs 18 mm wide, 16 mm high and 0·75 mm thick were used. Total acrylamide concentration was 4% in the stacking gel and 6% in the separating gel.

Three MHC isoforms (MHC I, MHC II A and MHC II B) were separated. Some examples of SDS–PAGE runs for single fibres are shown in Fig. 3.

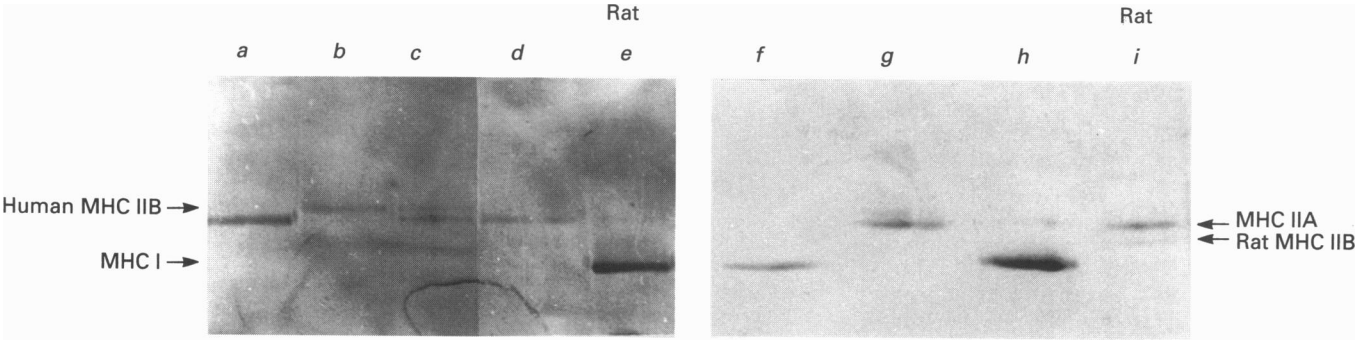


**Figure 3. SDS–PAGE analysis of MHC isoforms of single human skeletal fibres**

Lanes *a*, *b*, *d* and *f*–*h* refer to single human fibres from vastus lateralis muscle. Lane *c* refers to a homogenate of a bundle of fibres from human vastus lateralis muscle. Lanes *e* and *i* refer to single skeletal muscle fibres from rat plantaris muscle. Arrows indicate bands corresponding to MHC I, MHC II A, human MHC II B and rat MHC II B. Human and rat MHC I and II A comigrated.

## RESULTS

### MHC isoform composition

Several sets of fibres dissected from biopsy samples of human vastus lateralis muscles were studied in the present work. A group of fibres was subjected to load-clamp manoeuvres to obtain force–velocity curves. In a different group of fibres, $V_0$ was determined directly by the slack-test technique. Finally the temperature dependence of $V_0$ and $V_{max}$ was studied in still different groups. All fibres used were analysed for MHC isoform composition by SDS–PAGE. As shown in Fig. 3, in the area of migration of MHCs, SDS–PAGE could separate three bands corresponding to: MHC I (or slow isoform), MHC II A and MHC II B (or fast isoforms). At variance with what is observed in small mammals (Schiaffino & Reggiani, 1996), only three and not four MHC isoforms have been identified so far in human muscles (Biral, Betto, Danieli-Betto & Salviati, 1988; Larsson & Moss, 1993). Rat and human MHC I and MHC II A isoforms comigrate on SDS–PAGE gels. Rat and human MHC II A and MHC II B isoforms have a reverse order of migration: rat MHC II B migrates faster than MHC II A and human MHC II B slower than MHC II A. On the basis of MHC content, fibres have been grouped in five types: type I (or slow), type II A and type II B (or fast) containing MHC I, MHC II A and MHC II B, respectively; type I–II A and type II A–II B show co-existence of MHC I and MHC II A and of MHC II A and MHC II B, respectively. Of the total number of fibres studied ($n = 151$): 37% were type I; 36%, II A; 10%, II B; 9%, I–II A and 8%, II A–II B.

### Contractile properties and MHC isoforms

**Unloaded shortening velocity ($V_0$).** Maximum shortening velocity ($V_0$) was determined in fifty-seven fibres at 12 °C using the slack-test technique. Mean values (± S.D.) of $V_0$ for

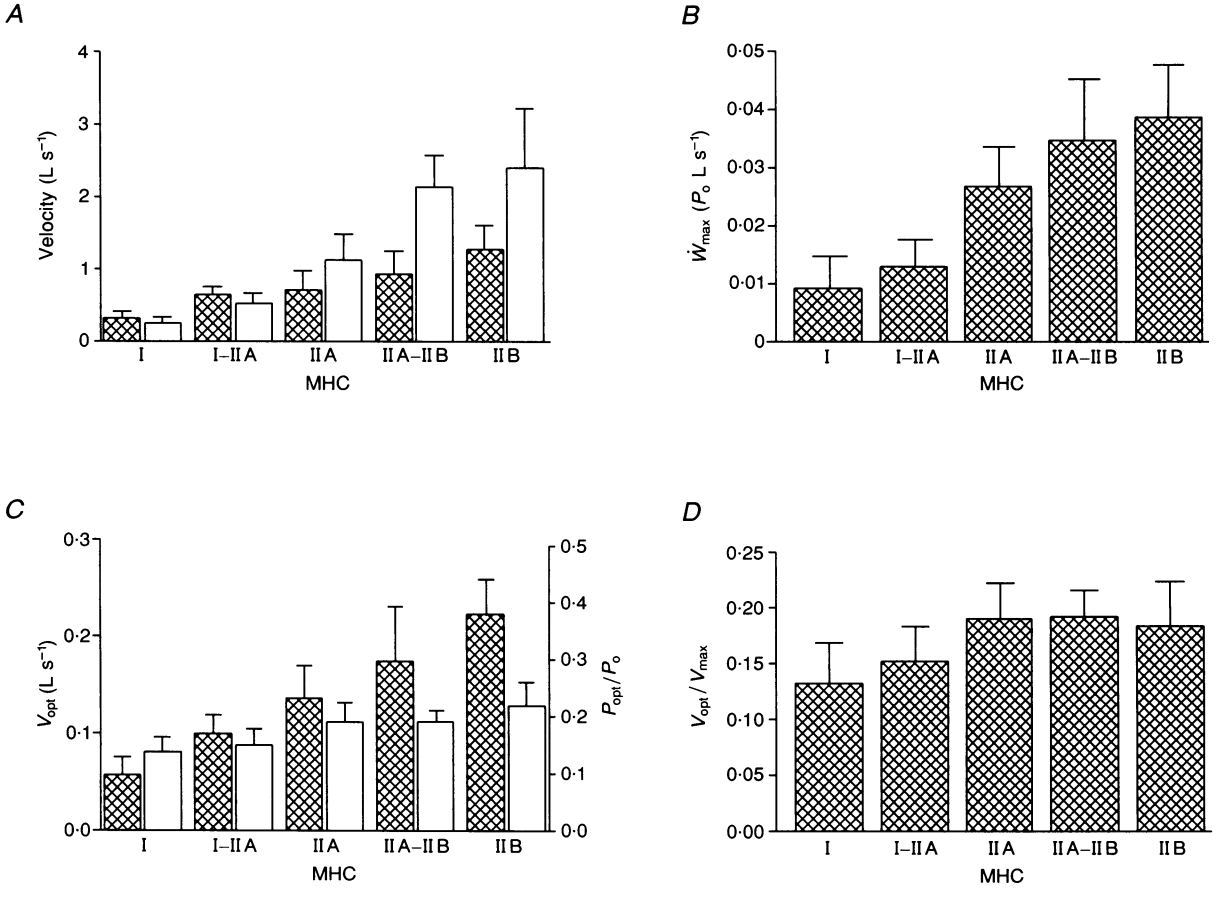


**Figure 4. $V_{max}$, $V_0$, $\dot{W}_{max}$, $V_{opt}$, $P_{opt}/P_0$ and $V_{opt}/V_{max}$ of five fibre types identified on the basis of MHC isoform composition**

The height of the columns represents the mean value of the parameters and the error bars are the S.D. In *A*: ▩, $V_{max}$; □, $V_0$; values for I, II A and II B fibres were significantly different, whereas values for I–II A and II A–II B were not significantly different from the corresponding 'pure' fibre types. In *B*, all differences were statistically significant. In *C*: □, $P_{opt}/P_0$; ▩, $V_{opt}$; differences were all statistically significant for $V_{opt}$ whereas for $P_{opt}$ only type I (I) and fast fibres (II A, II A–II B, and II B) were significantly different. In *D*, only the difference between type I and fast fibres was statistically significant. $V_0$, $V_{max}$ and $V_{opt}$ are in L s$^{-1}$; $P_{opt}$ is expressed relative to $P_0$; $\dot{W}_{max}$ is expressed relative to $P_0$ in $P_0$ L s$^{-1}$.

**Table 1. Maximum shortening velocity, specific tension and cross-sectional area**

| MHC | $V_o$ ($L s^{-1}$) | $n$ | $V_{max}$ ($L s^{-1}$) | $n$ | $a/P_o$ | $P_o^*/P_o$ | $P_o$/CSA ($kN m^{-2}$) | $n$ | CSA ($\mu m^2$) |
|---|---|---|---|---|---|---|---|---|---|
| I | 0·264 ± 0·089 | (24) | 0·317 ± 0·104 | (20) | 0·032 ± 0·024 | 0·991 ± 0·137 | 43·77 ± 21·90 | (53) | 9278 ± 3496 |
| I–II A | 0·521 ± 0·149 | (7) | 0·638 ± 0·121 | (6) | 0·030 ± 0·017 | 0·952 ± 0·052 | 50·97 ± 14·78 | (13) | 8569 ± 3211 |
| II A | 1·121 ± 0·361 | (20) | 0·718 ± 0·256 | (23) | 0·063 ± 0·029 | 0·994 ± 0·119 | 60·64 ± 34·86 | (43) | 7922 ± 2845 |
| II A–II B | 2·139 ± 0·453 | (2) | 0·936 ± 0·324 | (9) | 0·060 ± 0·016 | 0·997 ± 0·061 | 64·73 ± 14·48 | (13) | 5492 ± 1167 |
| II B | 2·418 ± 1·497 | (4) | 1·286 ± 0·316 | (9) | 0·072 ± 0·035 | 1·007 ± 0·091 | 61·84 ± 14·49 | (13) | 6294 ± 2159 |

Maximum shortening velocity determined by slack test ($V_o$) and from force–velocity curves ($V_{max}$), curvature of the force–velocity relationship ($a/P_o$), intercept of the force–velocity relationship with the force axis ($P_o^*/P_o$), specific tension ($P_o$/CSA) and cross-sectional area (CSA) of single human skinned muscle fibres classified on the basis of their myosin heavy chain (MHC) isoform composition. Means ± S.D. at 12 °C are reported. $V_o$ and $V_{max}$ were significantly lower in type I than in type II A and in type II A than in type II B fibres. $P_o$/CSA and $a/P_o$ were significantly lower in type I than in fast (II A and II B) fibres. Number of fibres for $V_{max}$ apply also to $a/P_o$ and $P_o^*/P_o$, and for $P_o$/CSA also to CSA.

the five fibre types are shown in Fig. 4 and reported in Table 1. $V_o$ was significantly lower in type I fibres than in fast fibres, and among fast fibres was slower in II A than in II B fibres. Mixed fibres (I–II A and II A–II B) were intermediate between the corresponding 'pure' fibre types.

**Maximum shortening velocity ($V_{max}$) and shape of the force–velocity curve.** A population of sixty-seven fibres was subjected to load-clamp manoeuvres at 12 °C to obtain force–velocity relationships. Figure 5*A* shows representative force–velocity curves for one type I and one II B fibre. For each fibre type, Table 1 reports the mean values of the following parameters of the force–velocity curve: maximum shortening velocity ($V_{max}$, intercept with the velocity axis), curvature of the force–velocity relationship ($a/P_o$), predicted isometric force ($P_o^*/P_o$ intercept with the force axis). Mean $V_{max}$ values are also shown in Fig. 4*A*. Mean $V_{max}$ values (± S.D.) were significantly lower for type I than for II A fibres, and for II A fibres than for II B fibres. Mean $V_{max}$ values for type I–II A fibres were intermediate between values of I and II A fibres and of type II A–II B were intermediate between values of II A and II B fibres. The data of Table 1 and Fig. 4 allow a comparison between $V_o$ and $V_{max}$ values of the same fibre type. Differences between $V_o$ and $V_{max}$ were not statistically significant for type I, but were statistically significant for both II A and II B fibres. The ratio $V_o$: $V_{max}$ was 0·83 for type I, 1·56 for II A and 1·88 for II B fibres.

Curvature of the force–velocity relationship was significantly higher (lower $a/P_o$ values) for type I than for fast (II A and II B) fibres; among fast fibres, curvature was higher for II A than II B fibres although the difference was not statistically significant. Finally, the mean values of the intercept with the force axis ($P_o^*/P_o$) ranged between 0·99 ± 0·137 for type I fibres and 1·01 ± 0·09 for II B fibres, and were not significantly different in the different fibre types.

**Maximum power output and optimal shortening velocity.** The force–velocity characteristics reported above were used to calculate maximum power output ($\dot{W}_{max}$) and velocity at which $\dot{W}_{max}$ developed ($V_{opt}$ or optimal velocity) for all fibres (see Methods). The fraction of $V_{max}$ ($V_{opt}/V_{max}$) and $P_o$ ($P_{opt}/P_o$) at which $\dot{W}_{max}$ was produced was also calculated. Figure 4*B–D* shows the mean values (± S.D.) of $\dot{W}_{max}$ (expressed relative to $P_o$), $V_{opt}$, $P_{opt}/P_o$ and $V_{opt}/V_{max}$ for all fibre types. Mean values of $\dot{W}_{max}$ and $V_{opt}$ for all the five fibre groups were significantly different, with type I fibres having the lowest values and type II B fibres the highest. As regards $V_{opt}/V_{max}$ and $P_{opt}/P_o$, type I fibres had significantly lower values than fast fibres (II A and II B fibres), whereas among fast fibres values were similar and differences were not statistically significant. Differences in $V_{opt}$, $P_{opt}/P_o$ and $\dot{W}_{max}$ can also be observed in Fig. 5*A* where force–power curves and force–velocity curves of representative type I and II B fibres are superimposed, and in Fig. 5*B*. In Fig. 5*B* the mean velocity–power curves were obtained from the mean values of the parameters of Hill's hyperbolic equation for each fibre type. As can be seen, differences in $V_{opt}$ paralleled differences in $\dot{W}_{max}$ in type I, II A and II B fibres. As power is expressed in actual values, i.e. watts per litre, the very large difference in the capacity to produce mechanical work of the three fibre types can also be noticed.

**Specific tension.** Table 1 also reports the mean values of specific tension ($P_0$/CSA) and cross-sectional area (CSA) for all fibres studied. Type I fibres had significantly lower values of $P_0$/CSA than fast fibres. No significant difference in $P_0$/CSA was found among fast fibres. No significant difference was found for CSA.

**Variability of contractile properties and MHC content.** The results reported so far are consistent with the idea that MHC isoforms have an important role in determining most of the contractile properties. However, the distribution of several contractile properties in the fibre populations studied (Fig. 6) suggests that the mechanism modulating muscle performance deserves further consideration and might not solely rely on MHC isoforms. Two points deserve comment. First, notwithstanding the significant differences in mean values, $V_{max}$, $\dot{W}_{max}$ and $V_{opt}/V_{max}$ consistently varied among fibres containing the same MHC isoform, i.e. within each fibre type, although to a variable extent. Second, ranges of variation of $V_{max}$, $\dot{W}_{max}$ and $V_{opt}/V_{max}$ of different fibre types overlapped though to a different degree. When type I and fast fibres are considered, the overlap was small for $V_{max}$, but large for $V_{opt}/V_{max}$; no overlap was seen for $\dot{W}_{max}$. Among fast fibres (II A and II B fibres), ranges of variability of $V_{max}$, $\dot{W}_{max}$ and $V_{opt}/V_{max}$ largely overlapped. Similar large variability within the same fibre type and large overlap in variability ranges between different fibre types was found also for $V_0$ (not shown). By contrast, not only were mean $V_{opt}$ values of all fibre types including mixed types (I–II A and II A–II B) significantly different, but also hardly any overlap occurred between different fibre types. Only mixed fibre types were found to bridge 'pure' fibre type ranges of $V_{opt}$. Variability in $V_{opt}$ among fibres containing the same MHC was somewhat smaller than for $V_{max}$ and $\dot{W}_{max}$.

### Temperature dependence

A group of twenty-one fibres was subjected to two slack-test manoeuvres, each comprising four to five quick releases, for $V_0$ determination at two temperatures in the range 12–22 °C. $V_0$ was assessed at 12 °C ($n = 21$, control temperature) and either at 17 °C ($n = 11$) or at 22 °C ($n = 10$, test temperatures). The $V_0$ determination at test temperatures preceded the control measurement in half the fibres and followed the control measurement in the other half. To support, by an independent approach, the results on the temperature dependence of $V_0$, attempts were made to determine the temperature dependence of $V_{max}$, i.e. maximum shortening velocity obtained from force–velocity extrapolation. At 12 °C, human fibres proved to be very stable and able to withstand the two very long (2–3 min) activations needed to obtain two force–velocity relationships (Fig. 2*A*). When force–velocities were studied at 12 and 17 °C, fibres were less stable and often did not allow reliable $V_{max}$ determinations during the second activation. In only six fibres were force–velocity curves determined at 12 and 17 °C. It was not possible to extend the study of the temperature dependence of $V_{max}$ to 22 °C. For the same reason, it was not possible to extend the study of the temperature dependence of $V_0$ above 22 °C.

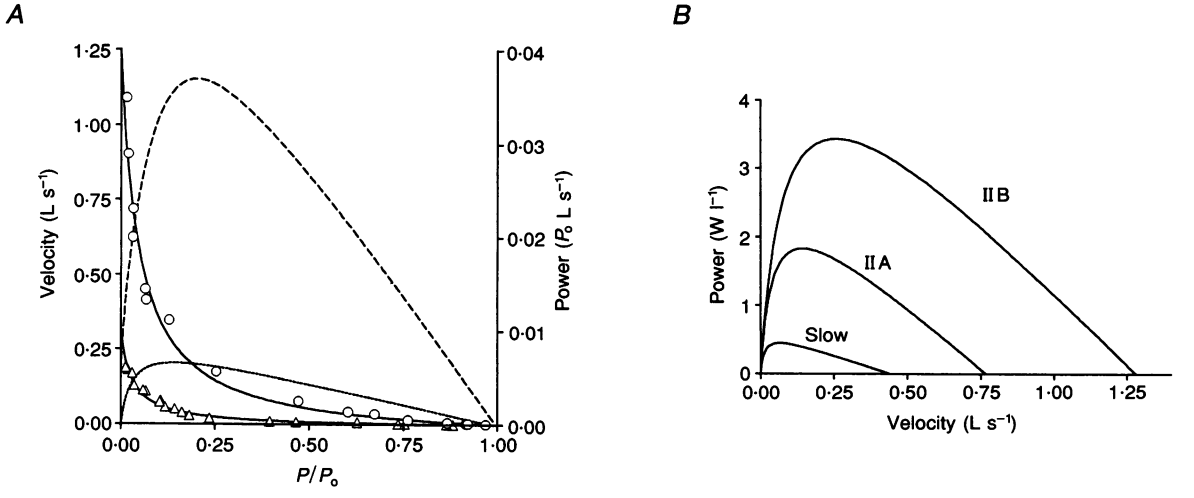


**Figure 5. Examples of force–velocity, force–power and velocity–power curves**

In *A*, force–velocity and force–power curves of a representative type I and II B fibre at 12 °C. The force–power curves are obtained from the parameters of the force–velocity curves. Velocity is expressed in L s$^{-1}$. Load and power are expressed relative to $P_0$, i.e. load as $P/P_0$ and power in $P_0$ L s$^{-1}$. Force–velocity curve: type I fibre, △; type II B fibre, ○. $V_{max}$ type II B fibre = 1·239 L s$^{-1}$; $V_{max}$ type I fibre = 0·309 L s$^{-1}$. Force–power curves: type I fibre, dotted line and type II B fibre, dashed line. In *B*, velocity–power curves obtained on the basis of the parameters of Hill's hyperbolic equation and of average specific tension for type I, II A and II B fibres at 12 °C. Power is expressed in absolute values, W l$^{-1}$.

Figure 7 shows examples of slack tests and force–velocity records at two temperatures. Figure 8*A* reports the mean values (± S.D.) of the ratios between $V_o$ at control and test temperatures (17 and 22 °C), and between $V_{max}$ at 12 and 17 °C. $V_o$ and $V_{max}$ were both very sensitive to temperature changes. $V_o$ was 5·96 ± 1·93 times higher at 22 than at 12 °C, and 2·86 ± 1·05 times higher at 17 than at 12 °C. $V_{max}$ was 2·47 ± 0·60 times higher at 17 °C than at 12 °C. The response to temperature changes was very variable from fibre to fibre as shown by the large standard deviations.

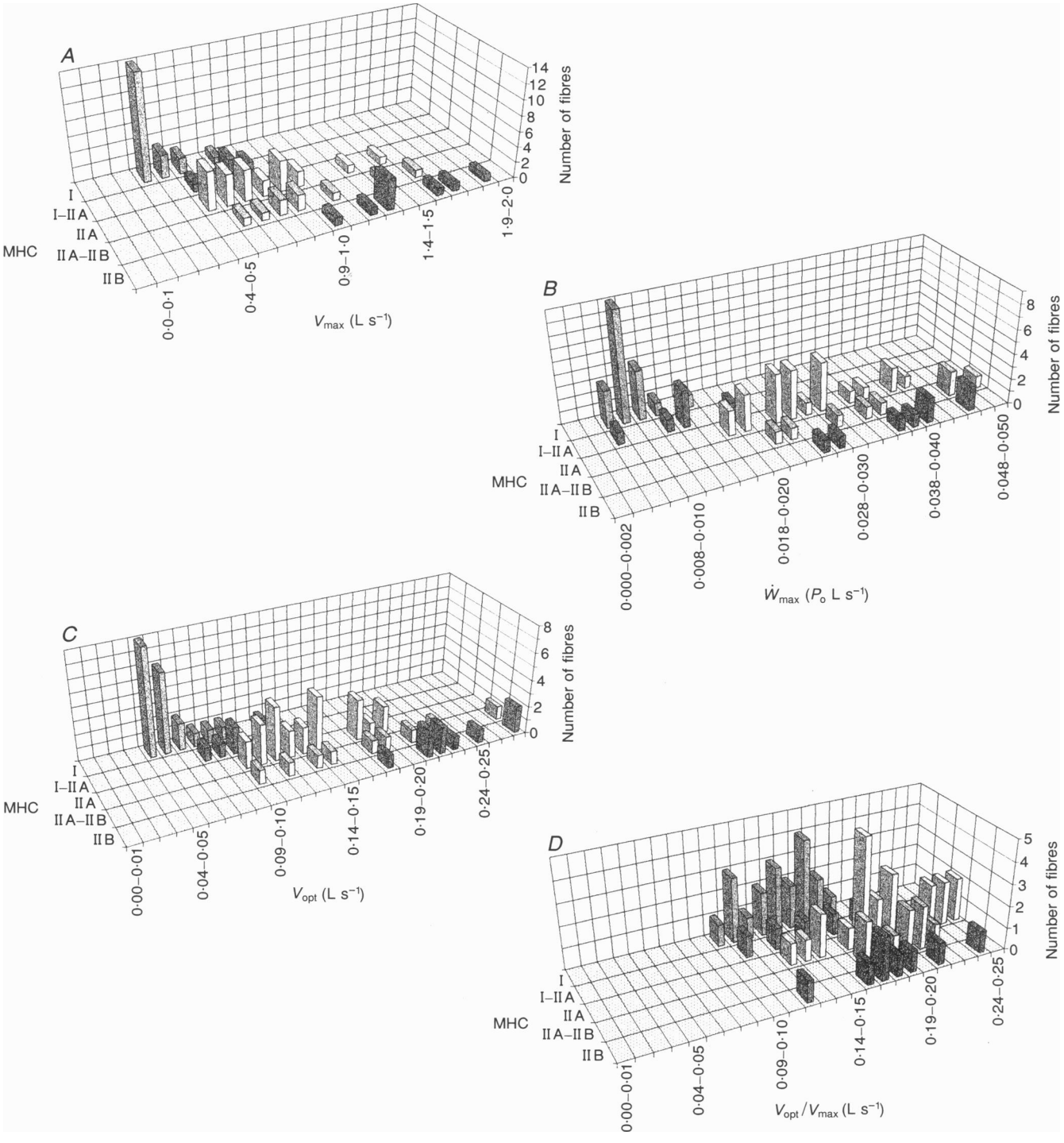


**Figure 6. Distribution of $V_{max}$, $\dot{W}_{max}$, $V_{opt}$ and $V_{opt}/V_{max}$ in the population of fibres studied**
Fibres are grouped on the basis of their MHC isoform composition determined by SDS–PAGE.

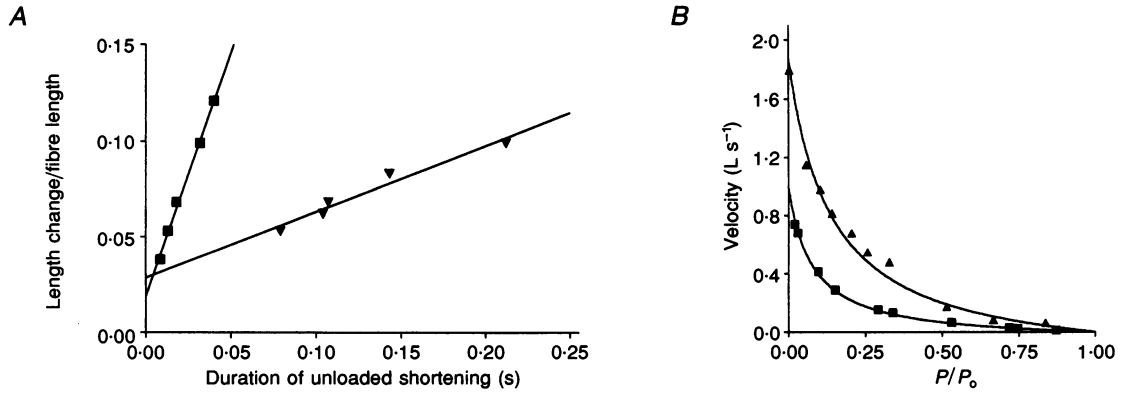


**Figure 7. Examples of slack-test data analysis and of force–velocity curves at two different temperatures**

In *A*, plot of slack-test data obtained from the same fibre (type I) at 12 °C (■), $V_0 = 0{\cdot}34$ L s$^{-1}$; and 22 °C (▼), $V_0 = 2{\cdot}55$ L s$^{-1}$. In *B*, two force–velocity curves obtained from the same fibre (type II A) at 12 °C (■), $V_{max} = 0{\cdot}981$ L s$^{-1}$; and 17 °C (▲), $V_{max} = 1{\cdot}871$ L s$^{-1}$.

The temperature dependence of isometric force ($P_0$) was determined in twenty-seven fibres. The mean values of the ratio between $P_0$ at control and test temperatures are reported in Fig. 8*A*. It can be seen that $P_0$ increased $1{\cdot}94 \pm 0{\cdot}73$ times from 12 to 17 °C ($n = 14$), but less between 17 and 22 °C (ratio $P_0$ at 22 °C/$P_0$ at 12 °C $= 2{\cdot}17 \pm 1{\cdot}2$, $n = 13$).

The $Q_{10}$ values and activation energy ($E$) of $V_0$ of human fibres were calculated using Arrhenius plots as described in Methods. The Arrhenius plots for $V_0$, $V_{max}$ and $P_0$ are shown in Fig. 8*B*. In this figure, mean values of the ratios between control and test temperatures are shown without standard deviations for sake of clarity. Standard deviations can be seen in Fig. 8*A*. The $Q_{10}$ (5·88) and $E$ (125 kJ mol$^{-1}$) values point to a very high temperature sensitivity of $V_0$. No regression analysis was performed on $V_{max}$. However, Fig. 8*A* and *B* shows that the value of the ratio of $V_{max}$ at 17 and 12 °C is very close to the corresponding value for $V_0$. No regression analysis was performed on $P_0$, as the Arrhenius plot for $P_0$ was not linear and clearly showed a plateau between 17 and 22 °C. Since all fibres used to study the temperature dependence of $V_0$ were also typed on the basis of MHC content, it was possible to consider separately the $Q_{10}$ and $E$ values of type I and fast fibres. $V_0$ of type I fibres ($n = 5$) showed apparently greater temperature dependence ($Q_{10}$, 6·98 and $E$, 136 kJ mol$^{-1}$) than $V_0$ of II A fibres ($n = 5$) ($Q_{10}$, 4·98 and $E$, 113 kJ mol$^{-1}$), but differences were not statistically significant. The same applied to the temperature sensitivity of $P_0$ that was apparently higher for type I than for fast fibres, but again the difference was not statistically significant.

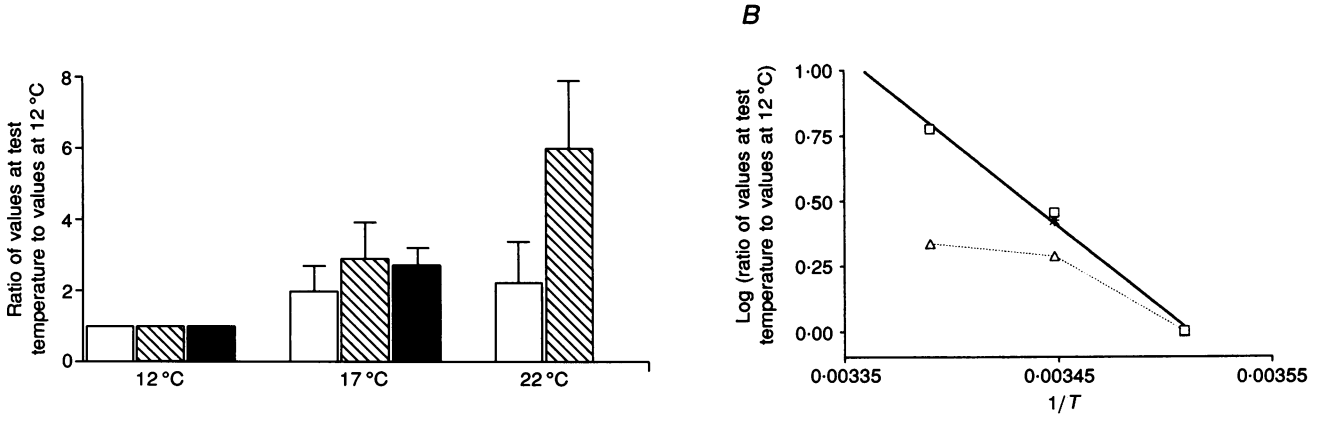


**Figure 8. Temperature dependence of $P_0$, $V_0$ and $V_{max}$**

In *A*, each column represents the mean values of the ratios between each parameter at test temperatures (17 and 22 °C) and at control temperature (12 °C). □, $P_0$; ▨, $V_0$; ■, $V_{max}$. Error bars represent S.D. $V_{max}$ was not studied at 22 °C. In *B*, Arrhenius plots for $V_0$ (□), $V_{max}$ (✳) and $P_0$ (△): $Q_{10}$, 5·88; $E$, 125 kJ mol$^{-1}$. The logarithm of the mean values of the ratios between each parameter at test and control temperature are plotted *vs.* temperature. S.D. are not shown for clarity. Temperature is expressed as $1/T$ where $T$ is absolute temperature. The continous line is the linear regression for $V_0$; the dotted line connects the values for $P_0$.

## DISCUSSION

In this study force–velocity properties of maximally activated single fibres from human vastus lateralis muscle were examined in great detail. Force–velocity properties ($V_o$, $V_{max}$, $V_{opt}$, $\dot{W}_{max}$, $a/P_o$, $P_o$/CSA) were tested for possible relationships with the MHC isoform composition of the fibres. $V_{max}$ and $V_o$ values of fibres containing the same MHC isoform were compared. The temperature sensitivity of $V_o$, $P_o$ and $V_{max}$ was assessed. The primary results of this study are that: (1) in human skeletal muscle fibres, MHC isoforms play an important role in modulating not only $V_o$ and $V_{max}$, but also $\dot{W}_{max}$ and $V_{opt}$, which are the key properties in determining muscle performance *in vivo*; (2) $V_{max}$ and $V_o$ values between fibres of the same type can be very different; $V_o/V_{max}$ ratios vary in different fibre types; and (3) maximum shortening velocity of human fibres is very sensitive to temperature changes in the range 12–22 °C, whereas $P_o$ is very sensitive to temperature changes between 12 and 17 °C, but much less sensitive between 17 and 22 °C.

### Contractile properties and MHC isoforms

In this study, as in previous studies (Biral *et al.* 1988; Larsson & Moss, 1993), three human MHC isoforms have been separated on SDS–PAGE gels. Three 'pure' (I, II A and II B) and two mixed (I–II A and II A–II B) fibre types have been identified. On the basis of previous observations on human fibres (Biral *et al.* 1988), the slowest migrating isoform on gels has been identified as MHC II B. More recent studies (Smerdu, Karsch-Mizrachi, Campione, Leinwand & Schiaffino, 1994; Ennion, Sant'Ana Pereira, Sargeant, Young & Goldspink, 1995) have suggested that human MHC II B could be analogous to rat MHC IIX isoform and not to rat MHC II B. Therefore human MHC II B should be more properly called IIX. However, for sake of simplicity, the usual, but probably less precise, nomenclature has been used in this work.

All contractile properties studied have been shown to depend on MHC isoforms although to a greatly variable extent.

The $V_o$ results confirm and the $V_{max}$ results, obtained by an independent approach, strengthen the idea that maximum shortening velocity of human fibres is mainly determined by their MHC isoform content (Larsson & Moss, 1993). This conclusion is in full agreement with similar studies on small mammals (Greaser, Moss & Reiser, 1988; Sweeney, Kushmerick, Mabuchi, Sreter & Gergely, 1988; Bottinelli *et al.* 1991, 1994*a*; Schiaffino & Reggiani, 1996), on large mammals (Rome, Sosnicki & Goble, 1990) and on amphibians (Edman, Reggiani, Schiaffino & te Kronnie, 1988) and points to an important role of MHCs in determining the rate of actomyosin interaction. However, as discussed below, it appears unlikely that MHC isoforms are the only determinant involved. With regard to absolute values, mean $V_o$ values of the different fibre types reported here are consistent with those reported by Larsson & Moss (1993) when the differences in working temperature (15 °C in Larsson & Moss, 1993, 12 °C in this study) and the large variability in this parameter are considered.

In this study, for the first time in human fibres, force–velocity properties such as maximum power output ($\dot{W}_{max}$), optimal velocity ($V_{opt}$) and the ratio $V_{opt}/V_{max}$ have been determined and tested for the relationship with MHC isoform composition. $\dot{W}_{max}$, $V_{opt}$ and $V_{opt}/V_{max}$ have been shown to be key characteristics of muscle contraction *in vivo* in fish. Combining *in vivo* and *in vitro* measurements in the carp, Rome *et al.* (1988) have demonstrated that *in vivo*: (1) slow and fast fibres shorten at different velocities, but both shorten at a velocity at which maximum power is developed and efficiency is optimized; and (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 and efficiency at all speeds. Since $V_{opt}$ and $V_{max}$ are closely related, and $V_{max}$ significantly depends on fibre type, the above mechanism requires the existence of different fibre types (Rome *et al.* 1988, 1990). In humans, combined *in vitro* and *in vivo* measurements and determination of fibre type recruitment during movement are not yet feasible. However, assuming that the mechanism of selective fibre type recruitment and of efficiency and power optimalization demonstrated by Rome *et al.* (1988) in the carp also holds true in humans, the results reported here can be readily fitted into a comprehensive framework. As human fibres have $V_{opt}$ values spanning a very large range (0·04–0·28 L s$^{-1}$, Fig. 6), according to the desired speed of movement, fibres with appropriate $V_{opt}$ could be selectively recruited to optimalize power and efficiency at all speeds and therefore the work produced and energy consumed. Since $V_{opt}$ increases with higher $\dot{W}_{max}$ (Figs 4 and 5), at increasing speed of movement more power is produced for any given number of fibres recruited. This is particularly evident when power is plotted *versus* velocity expressed in absolute values, i.e. in watts per litre (Fig. 5*B*). Also in humans $V_{opt}$ and $V_{max}$ are closely related (Fig. 4*D*) and depend on MHC isoform composition (Fig. 4*A* and *C*). Each fibre type covers a distinct portion of the entire range of $V_{opt}$ variability (Fig. 6). Therefore, different fibre types are necessary to optimize work and energy consumption at all physiological speeds. The difference in $V_{opt}/V_{max}$ between type I and fast (II A and II B) fibres, although much smaller than the difference in $V_{max}$ and insufficient to alter the above conclusions, deserve some comment, and will be discussed below.

Curvature of the force–velocity curve ($a/P_o$) and intercept with the force axis ($P_o^*/P_o$) describe the overall shape of the relationship. The $a/P_o$ values reported here for human skinned fibres are lower (curvature higher) than those previously observed for rat skinned fibres at the same temperature (Bottinelli *et al.* 1991), which in turn are lower than those reported for frog skinned fibres at 0–2 °C (Lou & Sun, 1993). Such differences in curvature of the

force–velocity relationship between species are so far mostly unexplained. The difference in the curvature of the force–velocity relationship between type I and fast fibres and, although not significant, between type II A and type II B fibres, can affect $V_{opt}/V_{max}$ values, $V_o/V_{max}$ ratio, and differences in $V_{max}$ between different fibre types. Since for higher curvatures $V_{opt}/V_{max}$ is lower, the lower $V_{opt}/V_{max}$ values of type I fibres might be determined by differences in curvature. Differences in curvature along with the known deviation of the force–velocity relationship from the hyperbolic shape at low loads (Julian *et al.* 1986) might explain why the $V_o/V_{max}$ ratio is higher in type II B fibres than in type I fibres and the differences in $V_o$ between fibre types are more clear cut than differences in $V_{max}$. $P_o^*/P_o$ values significantly different from values at 1·0 have been shown in several studies on intact frog fibres (Edman, 1988) and this was taken to suggest departure from the expected hyperbolic shape of the force–velocity relationship at high loads. In skinned fibres contradictory results have been obtained on this point (Julian *et al.* 1986; Bottinelli *et al.* 1991; Lou & Sun, 1993). $P_o^*/P_o$ values not significantly different from 1·0, as in this study, suggest that, at least under the conditions used, the force–velocity relationship did not depart significantly from the hyperbolic shape at high loads.

The present results suggest that in humans type I fibres develop less force than fast fibres. This is in full agreement with a very recent report on human fibres (Stienen *et al.* 1996). In another previous study (Larsson & Moss, 1993), specific tension showed similar variation with MHC isoforms in freeze-dried human fibres, but showed no such relationship in chemically skinned human fibres. With regard to the absolute values of $P_o$, it should be pointed out that the fibre cross-sectional area was much higher and $P_o$/CSA much lower in this study (at 12 °C) than in the previous study by Larsson & Moss (1993; 15 °C). Besides some temperature effect on $P_o$, the discrepancy might be due to the different procedures adopted to measure CSA and to the fact that no correction for fibre swelling was done in this study.

### Variability of contractile properties and MHC composition

The large variability of $V_o$, $V_{max}$, $\dot{W}_{max}$ and to a lesser extent $V_{opt}$ in fibres containing the same MHC isoform suggests that the mechanism modulating fibre performance might not rely only on MHC isoforms.

In small mammals, previous studies have shown that variability in $V_o$ and $V_{max}$ in different fibres containing the same MHC isoform cannot be explained by co-existence of different MHC isoforms in the same fibre (Bottinelli, Betto, Schiaffino & Reggiani, 1994*b*). On the contrary, myosin light chain (MLC) isoforms have been found to play a significant role. Variations in the relative content of the two alkali myosin light chain isoforms ($MLC_{1f}$ and $MyC_{3f}$) have been found to account for large variability in $V_o$ (Greaser *et al.* 1988; Sweeney *et al.* 1988; Bottinelli *et al.* 1994*a*) and $V_{max}$ (Bottinelli & Reggiani, 1995). In humans, the issue of whether MHC co-existence, or MLC content or both are involved in determining the large variability in $V_o$ and $V_{max}$ within the same fibre type is still unsettled. A fourth and still unidentified MHC isoform might exist and be co-expressed with the other isoforms (Smerdu *et al.* 1994; Ennion *et al.* 1995). With regard to MLC isoforms, it has been suggested that dithionitrobenzoic acid MLC isoforms ($MLC_{2s}$ and $MLC_{2f}$) could play a role whereas the importance of alkali MLC isoforms has not yet been demonstrated (Larsson & Moss, 1993). Additional work is required on both MHC isoform identification and MLC role to settle the issue.

The variability in $\dot{W}_{max}$ and $V_{opt}$ within the same fibre type has not yet been explained, either in small mammals that represent the most well-known model from this point of view or in humans. Alkali MLC isoforms do not seem to play a significant role in the rat (Bottinelli & Reggiani, 1995). The point is not of minor importance due to the physiological relevance of $\dot{W}_{max}$ and $V_{opt}$.

### Temperature dependence

Force developed in isometric conditions increased almost twofold between 12 and 17 °C and then little (about 11%) between 17 and 22 °C. This result is in agreement with previous observations in humans (Ranatunga, Sharpe & Turnbull, 1987; Stienen *et al.* 1996), small mammals (Ranatunga, 1982, 1984) and frog (Godt & Lindley, 1982).

This study reports the first determination of temperature sensitivity of maximum shortening velocity in human muscle fibres. The $Q_{10}$ (5·88) observed in this study is comparable to the value of 4·6 observed in trabeculae from rat heart in a recent study (de Tombe & Ter Keurs, 1990) in which precise $V_o$ determinations and maximum activation were done. A $Q_{10}$ value of 5·88 appears, however, very high when compared with $Q_{10}$ values between 2 and 3 obtained in intact whole muscles of the rat in the temperature range 10–35 °C (Ranatunga, 1982, 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). Such discrepancies between the values reported here for human skeletal fibres and the values previously found in rat and frog have plausible explanations: (1) the preparations used for $Q_{10}$ determination; (2) different temperature sensitivities of the species studied; and (3) variations in the temperature sensitivity depending on the temperature range studied. For example, different specimens were used in the studies of rat muscle (intact whole muscles) and in this study (single skinned fibres). The study of the temperature dependence of intact muscles might be hindered by lack of oxygen inside the specimen especially at high temperatures (Gibbs, 1978) and by difficulties in properly assessing $V_{max}$. Whole muscle $V_{max}$ measurements are, in fact, impaired by fibre heterogeneity (Claflin & Faulkner, 1989). The temperature sensitivity of the shortening velocity of skeletal muscles might differ

between species since there are differences in the range of temperatures at which muscles work in physiological conditions and the capacity to regulate body temperature. Muscle contraction might be less temperature sensitive in amphibians than in small mammals (Bennett, 1984), and in small mammals than in humans. Temperature dependence of shortening velocity has been shown to be higher at lower temperatures both in amphibians and in mammals (Bennett, 1984; Johnston & Sidell, 1984; Ranatunga, 1984; Pate *et al.* 1994). It is possible that each species is more temperature sensitive at temperatures different from its muscle physiological working temperature (Bennett, 1984). This could be more evident in humans which regulate body temperature within a very narrow range.

It appears unlikely that the high $Q_{10}$ and $E$ values reported here are due to some experimental artifact. The ionic composition of the solutions used at the different temperatures was adjusted to keep free ionic concentration, ionic strength and pH constant. It is remarkable that $V_{max}$ and $V_0$ obtained by an independent approach showed a very similar temperature dependence. Moreover, in the same fibres that displayed such a high temperature sensitivity for $V_0$ and $V_{max}$, the relationship between $P_0$ and temperature was found to be fully comparable to previous results (Stienen *et al.* 1996).

The $Q_{10}$ and $E$ values for maximum shortening velocity of this study are higher than $Q_{10}$ (2·6) and $E$ (63 kJ mol$^{-1}$) values of the ATPase activity of isolated single fibres measured in isometric conditions in the range 12–30 °C (Stienen *et al.* 1996), but comparable to those of the actin-activated ATPase activity of rat and rabbit myofibrils in solution (Siemankowski *et al.* 1985; $Q_{10}$, 5·64; $E$, 122 kJ mol$^{-1}$ in the rat). This might suggest, in agreement with de Tombe *et al.* (1990), that the rate-limiting step is the same for actomyosin interaction in solution and for unloaded shortening of isolated single fibres *in vitro*, whereas it is different for unloaded shortening and for isometric conditions. However, temperature sensitivity of actin-activated myosin ATPase activity in solution has not been determined so far in human muscle preparations.

In agreement with previous findings (Ranatunga, 1982, 1984), the results reported here suggest that both maximum shortening velocity and specific tension are more temperature sensitive in type I fibres than in fast fibres. However, differences between type I and fast fibres did not reach statistical significance and more work is needed to fully clarify this point.


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