

Energy turnover in relation to slowing of contractile properties during fatiguing contractions of the human anterior tibialis muscle

David A. Jones^{1,2}, Duncan L. Turner^{3,4}, David B. McIntyre¹ and Di J. Newham⁵

¹School of Sport and Exercise Sciences, University of Birmingham, Birmingham, UK

²Institute for Biomedical Research into Human Movement and Health, Manchester Metropolitan University, Manchester, UK

³School of Health and Biosciences, University of East London, London, UK

⁴Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK

⁵Division of Applied Biomedical Research, King's College London, London, UK

Slowing and loss of muscle power are major factors limiting physical performance but little is known about the molecular mechanisms involved. The slowing might be a consequence of slow detachment of cross bridges and, if this were the case, then a reduction in the ATP cost of an isometric contraction would be expected as the muscle fatigued. The human anterior tibialis muscle was stimulated repeatedly under ischaemic conditions at 50 Hz for 1.6 s with a 50% duty cycle and muscle metabolites measured by ³¹P magnetic resonance spectroscopy. Over the course of 20 contractions the half-time of relaxation increased from 36.5 ± 0.09 ms (mean \pm s.e.m.) to 113 ± 17 ms and isometric force was reduced to $63 \pm 3\%$ of the initial value. ATP turnover was determined from the change in high energy phosphates and lactate production, the latter estimated from the change of intracellular pH. ATP turnover over the first three contractions was 2.45 ± 0.09 mm s⁻¹ and decreased to 1.8 ± 0.06 mm s⁻¹ over the last five tetani. However, when this latter value was normalised for the decrease in isometric force, it became 2.56 ± 0.3 mm s⁻¹, which is the same as the turnover of the fresh muscle. The data suggest that the rate of cross bridge detachment is unaffected by fatigue and are consistent the suggestion that it is the rate of attachment which is slowed rather than the rate of detachment. The present results focus attention on stages in the cross bridge cycle concerned with attachment and the transition from low to high force states that may be influenced by metabolic changes in the fatiguing muscle.

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Corresponding author D. A. Jones: School of Sport and Exercise Sciences, University of Birmingham, Birmingham B15 2TT, UK. Email: d.a.jones@bham.ac.uk

Abbreviations AT, anterior tibialis; MRS, magnetic resonance spectroscopy; PC, phosphocreatine; PD, phosphodiester; PM, phosphomonoester.

Metabolically demanding exercise that results in fatigue produces shifts in contractile characteristics of the working muscles that are similar to the differences between fast and slow muscle fibres and this analogy could provide an insight into the change of kinetic properties that occurs during fatigue. One feature of fast and slow muscles in the fresh state is that they have different economies of ATP turnover during isometric contractions, economy being defined as the ATP turnover divided by the isometric force generated. The difference in economy is reported to be about threefold in isolated animal muscles (Goldspink, 1978; Crow & Kushmerick, 1982) and three- to fourfold for human and rat muscle fibres (Söderlund *et al.* 1992; Bottinelli *et al.* 1994; Szentesi *et al.* 2001). While there

are differences in the costs of calcium transport, etc., the major part of the differences in ATP costs between different fibre types can be ascribed to differences in cross bridge turnover. If, therefore, fatigue is analogous to a transition from a faster to a slower fibre type, the slowing of contractile properties might be associated with a decrease in ATP costs and an increase in economy of the muscle.

Edwards *et al.* (1975) first suggested that slow relaxation of fatigued muscle might be a consequence of slow cross bridge detachment and presented data on an isolated mouse soleus preparation that indicated a reduction in ATP turnover as the muscle fatigued and slowed. Crow & Kushmerick (1982) found no evidence of such a reduction in the soleus muscle although the experimental protocols

differed in the two studies. However the latter authors did find that the economy of the faster EDL increased over 15 s of stimulation. Subsequently they (Crow & Kushmerick, 1983) showed that the change in ATP turnover in the EDL correlated with changes in the maximum velocity of unloaded shortening, although values were measured at only two time points, 3 and 9 s. Other studies have been less successful in demonstrating a decrease in the energy costs of force generation during contractions that would be expected to result in substantial contractile slowing. In one of the first MRS studies of skeletal muscle, Dawson *et al.* (1980) found no change in the economy of frog muscle as it fatigued and, likewise, Hultman & Sjöström (1983) found no change in a biopsy study of human muscle stimulated at 20 Hz over 50 s. In another biopsy study of human muscle Chasiotis *et al.* (1987) found a tendency for economy to decrease during intermittent stimulation while for continuous stimulation there was a slight increase in economy in the last stage. However, while the difference in energy cost between the two protocols was significant in the latter stages, the changes with time within each protocol were not significant. Westra *et al.* (1988) compared isometric force and ATP costs in electrically stimulated rat quadriceps and found biphasic changes with time, first an increase in economy and then a decrease. More recently, Giannesini *et al.* (2001) measured the ATP costs of stimulating the rat gastrocnemius muscle at a range of frequencies from 0.8 to 7.6 Hz and related this to the force of the evoked twitches. With protocols that induced a loss of force, the ATP costs declined more than force and the apparent economy increased, but there was no correlation between the extent of fatigue and change in economy and, even with the protocols that produced no loss of force, there was still an improvement in economy with time. There is therefore some confusion as to whether fatigue-induced slowing of contractile properties is accompanied by a reduction in ATP costs of the contraction.

High intensity exercise is associated with a number of changes in contractile properties, the most evident being an increased time for relaxation from an isometric contraction (Edwards *et al.* 1975; Curtin, 1986, 1988; Cady *et al.* 1989*b*) and a change in the force–velocity relationship, including a decrease in the velocity of unloaded shortening (Crow & Kushmerick, 1983; de Haan *et al.* 1989; de Ruyter *et al.* 1999, 2000). While most attention has focused on changes in the maximum velocity of shortening, we have shown that the time course of the change in relaxation rate differed from that of the slowing of shortening velocity with the change in the latter being a relatively late event during fatigue and playing a minor role in the characteristic loss of power (Jones *et al.* 2006). What was notable and unexpected in that study was the fact that the curvature of the force–velocity relationship increased substantially with fatigue and this

not only accounted for most of the loss of power but it also correlated with the change in relaxation rate. The change in curvature is difficult to explain in terms of slower cross bridge detachment and the authors suggested that it was the apparent rate of cross bridge attachment which decreased, possibly as a consequence of increased levels of inorganic phosphate.

A decrease in the rate of cross bridge detachment should be evident as an increase in the economy of maintaining isometric force since a slowing of the rate of detachment would both increase force and decrease ATP turnover. On the other hand, a decrease in the rate of attachment would not be expected to affect economy since there would be concomitant decreases in both force and ATP turnover. Consequently we have undertaken a study of ATP turnover in electrically stimulated human anterior tibialis (AT) muscle and related this to changes in the rate of relaxation from an isometric tetanus, using this as an indication of contractile slowing that results in a loss of muscle power. Muscle metabolites were measured by ³¹P magnetic resonance spectroscopy (MRS) and the ATP turnover determined and expressed as a function of force, giving a measure of economy for the isometric contractions.

Methods

Subjects

Four healthy subjects (one female) aged 26–49, were studied after giving their informed consent to the experimental protocol, which conformed to the standards set by the latest revision of the *Declaration of Helsinki* and was approved by the University College Hospital Ethics Committee.

Force recording and electrical stimulation

Subjects lay supine with one leg extended in the bore of the MRS magnet (see below for details) and securely strapped to a Perspex, wood and aluminium cradle that positioned the belly of the AT within the uniform field of the magnet (see Constantin-Teodosiu *et al.* 1997; Fig. 1). The heel rested on a small cup fixed to a hinged wooden plate and the foot and toes were firmly strapped to the upper part of the plate that was attached to an aluminium strain gauge. A screw arrangement between footplate and transducer allowed adjustment of the angle of the foot plate and thus optimal muscle length. The MRS coil was positioned over the belly of the AT against the skin and adhesive ECG electrodes, used for stimulating the muscle, were placed just proximal and distal to the coil. The stimulating current was set 20% above that required to produce maximal tetanic force. Stimulation parameters

were 100 ms duration square wave pulses at 50 Hz and a current of ~ 50 mA.

Tetanic force at the end of each 1.6 s contraction was measured. The relaxation rate was taken as the half-time ($t_{0.5}$), in seconds, of the exponential phase (usually from 50 to 25% tetanic force). The speed of relaxation was also expressed as a rate constant, calculated as $0.693/t_{0.5}$.

Fatiguing procedure

The muscle was first rendered ischaemic by means of a sphygmomanometer cuff placed around the thigh and inflated to 200 mmHg. A period of 30 s elapsed between cuff inflation and the start of stimulation to deplete intramuscular stores of O_2 . The ischaemic muscle was then stimulated with 1.6 s contractions with a 1.6 s recovery interval between: stimulation was continued for 3, 10, 15 or 20 contractions on separate occasions. At the end of the stimulation period the electrode leads and those to the strain gauge were rapidly removed and muscle metabolites determined whilst the muscle remained ischaemic.

Metabolite measurements

^{31}P MRS spectra were collected before and after the ischaemic exercise using methods similar to those previously described by Cady *et al.* (1989a). Briefly, relative concentrations of muscle metabolites were measured using a spectrometer equipped with a 1.9 T superconducting magnet and a 21 cm clear bore. A 2.1 cm two-turn coil was tuned for ^{31}P (32.5 MHz) and 1H (80.3 MHz) at the centre of the magnetic field. Radio frequency pulses giving a 90 deg flip angle at the coil centre were used with an interpulse interval of 2.256 s. A small sphygmomanometer cuff was placed under the calf and was used to adjust the position of the muscle in relation to the coil, keeping it in firm contact with the skin. The position of the coil and whether it had moved could usually be judged at the end of the experiment from the position and depth of the indentation made in the skin.

Each composite spectrum comprised 128 free induction decays (total collection time, 4.8 min) which were subjected to Fourier transformation, phasing and

baseline correction. The areas for the resonance peaks of phosphomonoester (PM), inorganic phosphate (P_i), phosphocreatine (PC), phosphodiester (PD) and β ATP were calculated by integration of the area between pre-selected gates. The total MRS visible phosphorus was taken to be the sum of PM + P_i + PD + PC + ($3 \times \beta$ ATP) and the individual compounds were expressed as a fraction of the total. Individual metabolite peaks were corrected for partial saturation using factors derived from comparisons of the data obtained with the 2.256 s interval with that obtained with a 20.56 s interval.

Although the MRS collection time was 4.8 min there was always some delay in repositioning the leg and shimming the magnet so the time elapsed between the end of stimulation and the end of the collection was between 7 and 10 min.

β ATP/total P for the resting muscles was 0.125 ± 0.007 . The total P was calculated assuming the ATP concentration to be 8.2 mM in the intracellular water (Cady *et al.* 1989a) giving a total P of 65.9 ± 3.7 mM. This value was then used to estimate the absolute metabolite concentrations for the post-exercise measurements, making the assumption that the total visible P signal remained constant.

Intracellular pH was estimated from the chemical shift of the P_i peak from the PC peak:

$$pH = 6.75 + \log[(\delta - 3.27)/(5.69 - \delta)]$$

where δ is the chemical shift (ppm) of the P_i peak relative to PC.

Lactate production was calculated by assuming that it was equivalent to the protons required to produce the observed change of pH, making further assumptions about the concentrations and dissociation constants of the intracellular buffers. These buffers were taken to be: (a) protein-bound histidine, 56 mM, $pK = 6.8$ (Fürst *et al.* 1970); (b) bicarbonate, 10 mM, $pK = 6.1$ (Sahlin *et al.* 1977); (c) carnosine, 7 mM, $pK = 6.8$ (Mannion *et al.* 1992); and (d) phosphate, added to the computation according to the phosphate measured by MRS ($pK = 6.73$). The non-phosphate buffer capacity came to 34.5 slykes, and this increased to approximately 50 slykes when P_i reached its highest levels in the fatigued muscles. The free intracellular ADP concentration was

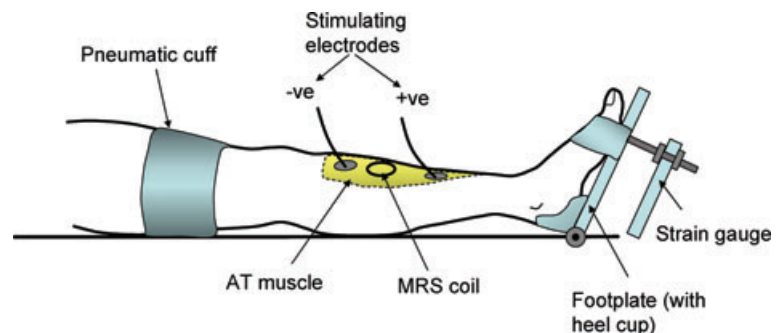


Figure 1. Diagram of the experimental arrangement for stimulating and measurement of metabolites from the anterior tibialis muscle

Subjects lay with their leg in the bore of the magnet and the belly of the muscle positioned within the uniform magnetic field.

calculated from the concentrations of ATP, PC and H^+ assuming the total creatine and phosphocreatine remained constant throughout the exercise at 42.5 mM:

$$[ADP] = ([42.5 - PC][ATP])/([PC][H^+]K_{eq}).$$

The equilibrium constant (K_{eq}) for the reaction was taken to be $1.66 \times 10^{-9} M^{-1}$.

The concentration of the monobasic phosphate was estimated from the total P_i and pH: $[H_2PO_4^-] = ([H^+][P_i])/(K_p + [H^+])$.

The equilibrium constant K_p was taken to be $1.86 \times 10^{-7} M^{-1}$.

ATP cost was estimated from changes (Δ ; post-contraction – pre-contraction) in PC, ATP and LA concentrations:

$$\Delta ATP = (1.5 \times \Delta LA) - (\Delta PC) - (\Delta ATP)$$

Data handling

Four measurements were made for each subject so for each there were four determinations of resting metabolite levels and one at the end of 3, 10, 15 and 20 tetani. The four resting measurements for each subject were averaged to provide a single value which was then used to determine the change as a result of stimulation. The force records yielded four values for force and relaxation for each subject for fresh muscle and for the first three tetani, four values for the next five, three for the next five, and so on. As with the metabolites, values for each subject were averaged at each time point to give a single value to be combined with the data from the other subjects. ATP turnover is reported as $mM s^{-1}$, this being millimoles ATP per litre intracellular water per second. Values are given as means and standard errors of the mean unless stated otherwise.

Results

The stimulation protocol was well tolerated by the subjects, the only problem being the ischaemic interval at the end of stimulation, which became a little tedious while adjustments were being made and MRS data collected. However the only ill effects were attacks of pins and needles once the blood returned to the limb.

Changes in force and contractile properties with fatigue

Peak force (expressed as a percentage of the value of the first tetanus) for the series of 20 contractions is shown in Fig. 2A. There was a suggestion of a biphasic response with a fairly steady decline over the first 20 s of stimulation (12–13 tetani) with a somewhat faster decline thereafter. At the end of the protocol tetanic force was reduced to $63 \pm 3\%$ of the initial value.

Relaxation half-time (Fig. 2B) changed relatively little over the first 15 s of stimulation (9–10 tetani) but then increased rapidly during the second half of the protocol. Values for the fresh muscle were 36.5 ± 0.9 ms and these increased to 113 ± 17 ms by the end of the protocol, just over a threefold increase. Expressed as rate constants, the values declined from $19 \pm 0.5 s^{-1}$ in the fresh muscle to $6.7 \pm 1.2 s^{-1}$ at the end the fatiguing series. The point of inflection for the relaxation–time relationship corresponded roughly with the time at which force began to decline more rapidly (compare Fig. 2A and B).

Muscle metabolites

Muscle metabolites measured at rest and after 3, 10, 15 and 20 fatiguing tetani are given in Table 1 and shown in Fig. 3. Values are reported as concentrations in the intracellular water and are normalised to 8.2 mM in the

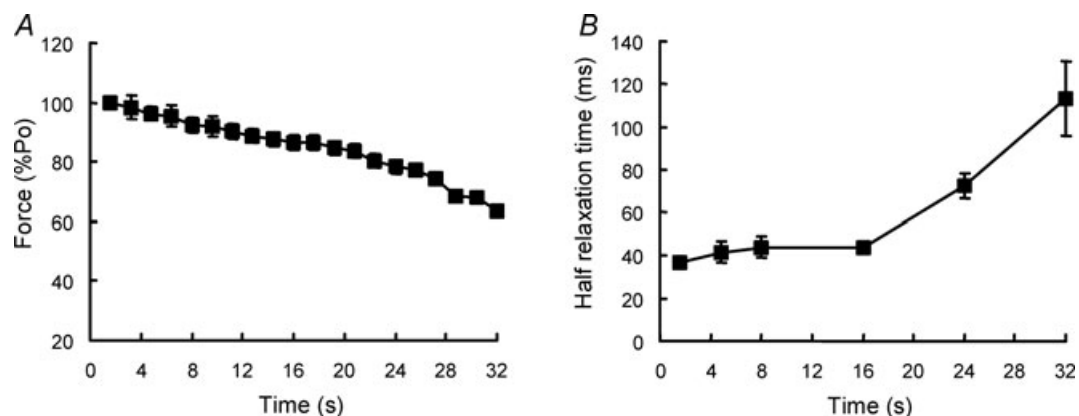


Figure 2. Change in contractile properties with fatigue

The ischaemic muscle was stimulated repeatedly at 50 Hz for 1.6 s with 1.6 s rest. Force (A) is expressed as a percentage of the initial isometric contraction (P_0) and the rate of relaxation (B) is expressed as the half-time of force decay.

Table 1. Muscle metabolites

Time (s)	0	4.8	16	24	32
ATP	8.2 ± 0.0	7.6 ± 0.8	7.6 ± 0.2	6.9 ± 0.7	7.0 ± 0.4
PC	32.3 ± 2.4	22.2 ± 2.4	14.4 ± 1.4	9.5 ± 1.6	7.8 ± 1.6
P _i	5.6 ± 0.2	14.0 ± 5.3	22.4 ± 4.3	25.4 ± 3.6	29.6 ± 2.2
ME	3.3 ± 1.1	3.6 ± 0.6	6.9 ± 1.5	9.4 ± 2.2	8.4 ± 1.2
pH	7.01 ± 0.02	7.09 ± 0.02	6.90 ± 0.02	6.78 ± 0.05	6.65 ± 0.05
Lactate	0	0.7 ± 0.9	12.1 ± 1.1	19.4 ± 3.6	28.0 ± 3.6

The anterior tibialis was stimulated for the times indicated and metabolites measured in the ischaemic muscle by ³¹P MRS. Values are given as mmoles per litre intracellular water, assuming an ATP concentration of 8.2 mM in the resting muscle (0 time stimulation) and a constant total MRS visible P thereafter. ME, phospho-monoesters. Data are given as means and s.d.

fresh muscle and, thereafter, to a constant total phosphate (see Methods).

ATP levels were unaffected for the first 10 contractions but thereafter declined slightly, falling to 7.0 ± 0.2 mM from the initial value of 8.2 mM. Phosphocreatine declined rapidly from a starting value of 32.3 ± 1.2 mM to a final value of 7.8 ± 0.8 mM (Δ PC -24.5) and this was matched by a stoichiometric rise in P_i from 5.6 ± 1 mM to 29.6 ± 1.1 mM (Δ P_i 24.0). There was a rise in phospho-monoesters, due to accumulation of AMP or IMP as a consequence of the loss of ATP, but this peak would also include sugar phosphates and other glycolytic intermediates.

Intracellular changes in hydrogen ion are shown in Fig. 4A, and the corresponding pH values in Table 1. In the first 5 s there was a small alkalosis as a consequence of PC breakdown, after which pH declined in a linear fashion. Resting muscle pH was 7.01 ± 0.01 which declined to 6.65 ± 0.03 by the last contraction.

Lactate accumulation was calculated from the changes of intracellular hydrogen ion concentration (Fig. 4A) and the estimated buffer capacity of the muscle and it can be seen that there was virtually no evidence of glycolysis during the first 5 s of contraction (3 tetani: Fig. 3), and thereafter lactate increased in a linear fashion to reach 28.0 ± 1.8 mM by the last contraction.

ADP concentrations were estimated from the levels of ATP, PC and H⁺, assuming that the total creatine remained constant, and the values are given in Fig. 4B. After an initial increase the concentration appeared to stabilise at around 80 μM.

Muscle metabolites and relaxation

There were no simple relationships between change in any of the metabolites and the slowing of relaxation, largely due to the fact that the major change in contractile speed occurred relatively late in the series of contractions while the major changes in metabolites occurred early in the process. The changes in total ATP were small and although

levels continued to fall throughout the series there was no increased loss towards the end of the series that might have correlated with the slowing of relaxation. After an initial fall, hydrogen ion concentration rose steadily with increasing duration of stimulation and, at the higher concentrations, there was a linear relationship between relaxation and hydrogen ion concentration (Fig. 5A), but this certainly did not apply over the whole pH range. The relationship between inorganic phosphate (P_i) and rate of relaxation (Fig. 5B) was also biphasic with small changes in relaxation over the period when P_i was changing rapidly and more rapid slowing of relaxation in the latter stages. Inorganic phosphate exists as the dibasic (HPO₄²⁻) and the monobasic form (H₂PO₄⁻), with the proportion of the monobasic form increasing as the intracellular H⁺ concentration increases. The relaxation rate showed a more linear relationship with the monobasic phosphate (Fig. 5C) than with the total P_i, although the uncertainty becomes greater since the estimates of

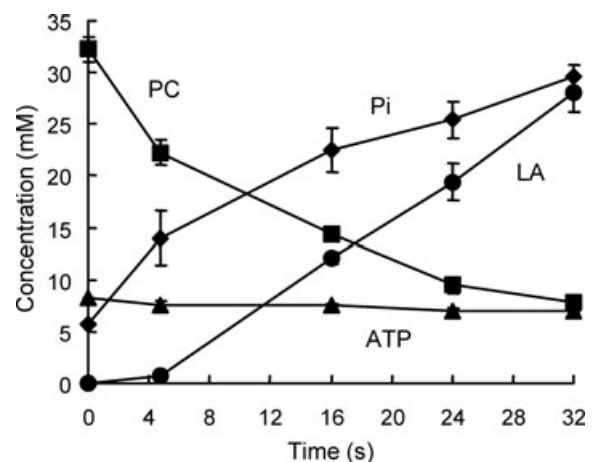


Figure 3. Changes in ATP, phosphocreatine (PC), inorganic phosphate (P_i) and lactate (LA) estimated by ³¹P MRS, during the course of the fatiguing series of contractions illustrated in Fig. 2.

Concentrations are given as mmoles per litre intracellular water, assuming an ATP concentration of 8.2 mM in the resting muscle.

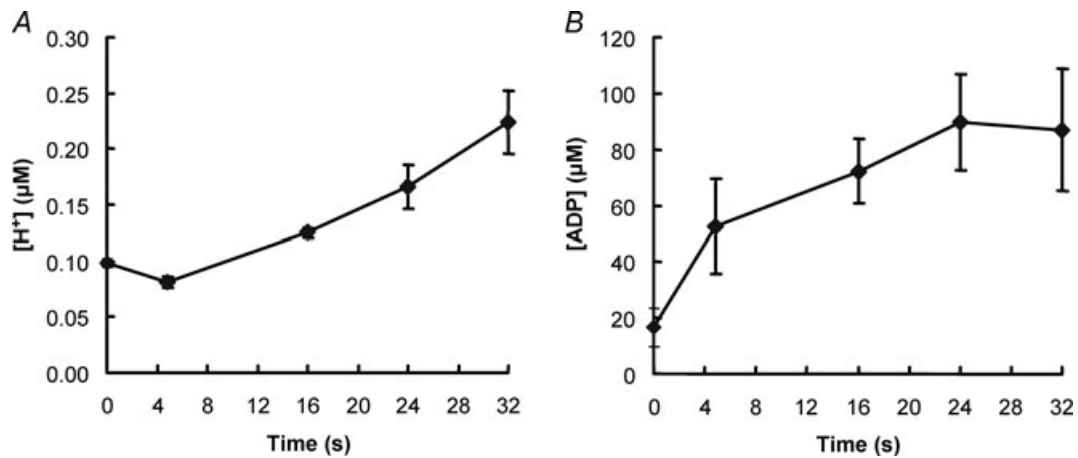


Figure 4. Changes in intracellular pH (A) and ADP (B) with fatigue
Experimental details as described for Fig. 3.

monobasic phosphate combine the errors involved in measuring both P_i and H^+ . ADP might affect cross bridge function in the fatiguing muscle but it appears unlikely that there is any causal relationship between ADP and rate of relaxation (Fig. 5) since ADP levels were constant at the time when relaxation rates were changing rapidly.

ATP turnover

ATP turnover was calculated for each subject on the basis of change in ATP, PC and $1.5 \times$ lactate. The change in total ATP turnover at the end of each period of stimulation (0, 4.8, 8, 16, 24 s) was subtracted from the

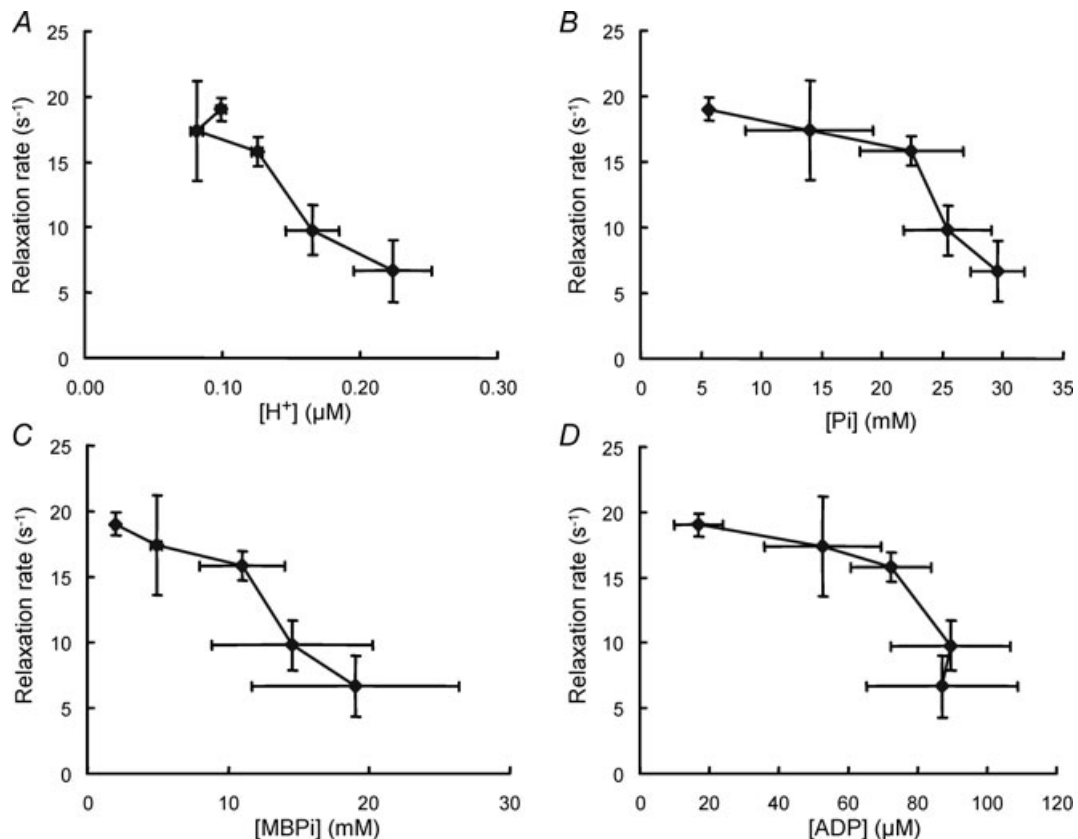


Figure 5. Muscle metabolite concentrations and relaxation

Relaxation rate in relation to: A, hydrogen ion concentration; B, inorganic phosphate (P_i); C, ADP; and D, monobasic phosphate (MBPi) after different periods of stimulation. Experimental details as described for Fig. 3. Data given as means \pm S.D.

values calculated for the next longest period (4.8, 8, 16, 24, 32 s respectively) and the results plotted as a mean value at the average time between these two times (2.4, 10.4, 20 and 28 s) in Fig. 6. The initial ATP turnover was $2.45 \pm 0.09 \text{ mM s}^{-1}$ during the first three contractions and this declined to $1.8 \pm 0.6 \text{ mM s}^{-1}$ over the last 5 tetani. However it should be noted that during this time the tetanic force also declined (Fig. 2) and, if the values for ATP turnover are normalised for loss of force (Fig. 7), no decrease in turnover was evident over the course of the fatiguing contractions, the corrected turnover for the last five contractions being adjusted to $2.56 \pm 0.8 \text{ mM s}^{-1}$.

Discussion

The main purpose of this study was to determine whether there is a change in the economy of an isometric contraction as the muscle fatigues and its contractile properties become slower. A slow relaxation has been associated with a loss of power and this might suggest a slower rate of cross bridge detachment that would result in an improved economy. However, the observation is that the economy was unchanged, a result that is consistent with the suggestion (Jones *et al.* 2006) that it is the rate of cross bridge attachment that changes with fatigue.

Unlike in many animals, the human AT is a relatively slow muscle, probably because it plays an opposing role to the soleus muscle in maintaining an upright posture. Resting metabolite levels and contractile properties, however, were very similar to those of the hand muscles, the first dorsal interosseous (FDI) (Cady *et al.* 1989a) and adductor pollicis (Newham *et al.* 1995). The stimulated rate of ATP turnover found in the present work of approximately 2.5 mM s^{-1} was similar to that reported

by Sacco *et al.* (1994), but about half the rate found for the adductor pollicis during a 5 s continuous tetanus (Newham *et al.* 1995).

The time course and extent of force loss were similar to those seen with other human muscles fatigued in a similar fashion. The relaxation phase at the end of each tetanus slowed with the onset of fatigue, the half-relaxation time being three times the value in the fresh muscle. The extent of the change was somewhat less than the fourfold change reported by de Ruiter *et al.* (2000) and Jones *et al.* (2006) for the adductor pollicis, but the difference is probably explicable in terms of the differing stimulation protocols, since the shortening contractions used in the previous studies would have been more energetically demanding than the isometric contractions used in the present work.

The measurement of metabolites by *in vivo* ^{31}P MRS makes a number of assumptions and has some practical limitations. The raw MRS data give values for the phosphorous metabolites as ratios and in converting these to absolute concentrations we have made two assumptions. The first is that the intracellular ATP concentration is 8.2 mM in the fresh muscle, while the second is that the total MRS visible phosphate remains constant throughout the series of fatiguing contractions. The first assumption will affect the absolute value of the ATP turnover but will not affect the conclusion that the rate of turnover does not change with duration of activity. If the total visible P was to decrease this would have the effect of reducing the apparent change in PC and ATP while accentuating changes in P_i . Lactate production is the major determinant of ATP turnover in the latter stages of the exercise and phosphate is a significant component of the total buffer capacity. Consequently overestimating phosphate will overestimate the lactate production. However, any such overestimate

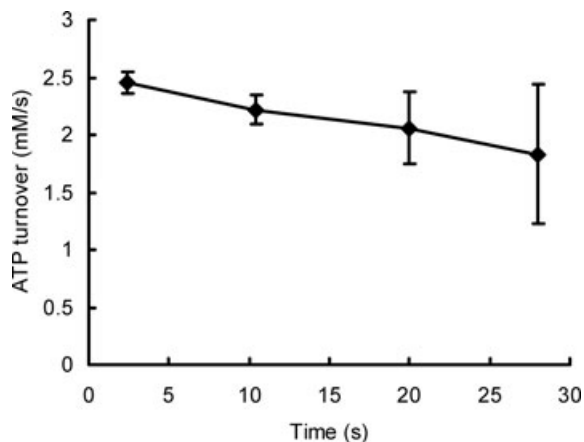


Figure 6. ATP turnover

Turnover was calculated from the data shown in Fig. 3 as the change in high energy phosphates plus the ATP from glycolysis estimated from lactate accumulation. Values are plotted against the average time over which the estimates were made.

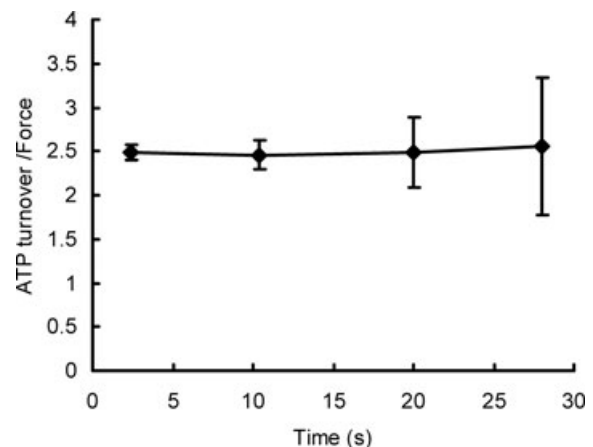


Figure 7. Economy of force production

Economy of isometric force production during the fatiguing series of contractions estimated by normalising the data shown in Fig. 6 by the force data in Fig. 2A.

would almost exactly be balanced by an underestimation of PC utilisation. In any case, it is unlikely that there is a significant loss of total visible P since changes in PC and P_i were almost exactly equal and opposite. MRS is a valuable technique in that it allows repeated measurements to be made on the same muscle but has the disadvantage of being relatively insensitive so that a number of spectra have to be averaged. In the system we used it was necessary to collect spectra over 4.8 min to obtain sufficient resolution of the major peaks. The technique is also sensitive to small changes in the position of the muscle in relation to the volume of the homogeneous field of the magnet. Consequently it was not possible to make concurrent force and metabolite measurements and we adopted the technique of stimulating the muscle and then measuring metabolites whilst the muscle was maintained under ischaemic conditions. The assumption here is that no further metabolic change occurs during the time from the end of stimulation to collection of the final spectrum, which could be up to 10 min. However resting metabolic rates of skeletal muscle are very low compared to the rates during contraction. We have found that the resting ATP turnover for the adductor pollicis is of the order of 0.01 mM s^{-1} compared to 5.5 mM s^{-1} during a tetanus (Turner, Jones & Newham, unpublished observations). It might be argued that the resting rate would be significantly greater following a period of activity, but Crowther *et al.* (2002a,b) have shown that glycolysis turns off rapidly at the end of contractile activity. In either case, any resting metabolism will make an equal contribution to all our measurements and since the rates of turnover were calculated by subtracting one value from another, a constant additional component will not contribute to the difference and thus the ATP turnover reported. One further assumption is that the muscle was working entirely anaerobically. Thirty seconds was allowed between making the muscle ischaemic and the start of the experiment, but this may not have been sufficient time to completely deplete O_2 stores in the muscle and would mean a higher ATP turnover than was calculated. However, it is likely that any residual oxygen would be used rapidly at the start of the contractile activity (Harris *et al.* 1975) and any uncertainty on this point should not affect conclusions about the economy of the longer contractions when the major changes in contractile properties occurred.

Although cross bridge activity may be the major process using ATP, it is also required for calcium pumping and maintaining electrochemical gradients. The energy requirements of these latter processes might be expected to remain relatively constant, especially if the ATP turnover is corrected for a decrease in force, assuming this is largely the result of a decrease in calcium release. The proportion of the total ATP consumed by cross bridge activity is not known for the human AT muscle but it is likely to be

30–40%, as for other muscle preparations (Homsher & Kean, 1978; Crow & Kushmerick, 1983; Szentesi *et al.* 2001). If, then, the rate of cross bridge ATP turnover were to be halved, a reduction of perhaps 30% of the total turnover might be expected. The data shown in Fig. 6 suggest a 20% decrease in turnover, but no evidence of a decrease is seen if a correction is made for force loss (Fig. 7).

We have not been able to measure the force–velocity relationships of the AT muscle since this is a technically demanding measurement to make on human muscle *in situ*, especially within the NMR magnet, but it is reasonable to infer that the curvature of the relationship would have changed in a similar way to that seen with the adductor pollicis in previous studies where the slowing of relaxation correlated well with changes in curvature (Fig. 4C in Jones *et al.* 2006). Using the same argument, it also seems likely that in the present experiments V_{max} might have changed relatively little since change in shortening velocity is a smaller and later feature of the fatigue process (Jones *et al.* 2006).

The slowing of contractile properties with fatigue has been interpreted as an indication of slow cross bridge detachment (de Haan *et al.* 1989; de Ruiter *et al.* 1999, 2000). However, the subsequent observation that changes in relaxation and loss of power were associated with an increased curvature of the force–velocity relationship (halving of a/F_0 , see Jones *et al.* 2006) has changed that view. In a two-state model of cross bridge action (Huxley, 1957), a/F_0 is determined by $(f + g_1)/g_2$, where f is the rate constant for attachment, g_1 that for detachment within the positive working range of the cross bridge and g_2 the rate constant for detachment once the cross bridge has passed the neutral position. The latter constant (g_2) determines V_{max} . In the absence of a major change in V_{max} , and thus g_2 , a decrease in a/F_0 implies a decrease in $(f + g_1)$. Values for f are generally assumed to be three- to fourfold higher than for g_1 to allow a reasonable proportion of cross bridges to be attached during an isometric contraction, and consequently it is impossible to account for a twofold change in $(f + g_1)$, as required to explain the reported decrease in a/F_0 with fatigue, by changing g_1 alone. It was argued, therefore, that to account for the changes in curvature of the force–velocity relationship with fatigue a major change in f is required, implying a decrease in the rates of cross bridge attachment rather than the previously held view that it was the rate of detachment that changed. In the cross bridge model of Huxley (1957), and in more recent models of muscle during slow shortening or in the isometric state (e.g. Piazzesi & Lombardi, 1995), each cross bridge cycle is associated with the hydrolysis of one ATP, so the ATP turnover per unit force in the isometric state (economy) gives a measure of g_1 , the rate constant for detachment. Our present observation of a lack of change in economy over the course of a series of fatiguing

contractions (Fig. 7) indicates that g_1 does not change and, by implication, any decrease in a/F_0 is due to a decrease in f , the rate constant for attachment.

There is then the question of whether a decrease in the rate constant for cross bridge attachment accounts for the slowing of relaxation from an isometric tetanus. Slow relaxation could be due to a change in cross bridge kinetics or slow Ca^{2+} removal from troponin into the sarcoplasmic reticulum. Although evidence from the use of mammalian preparations indicates that slowing with fatigue is primarily due to a change in cross bridge kinetics (Westerblad & Allen, 1993; Westerblad *et al.* 1997) this has not been established for human muscle working at body temperature. Assuming, for the moment, that cross bridge action does determine the rate of relaxation, it is still not immediately obvious how a decrease in attachment might slow the rate at which force decays. In single fibres the relaxation phase is characterised by an initial, relatively slow, linear phase of force decline followed by a shoulder and then a more rapid exponential decay of force. The shoulder, and subsequent loss of force, is associated with relative movements of different elements of the fibre, generally with the end sarcomeres being stretched (Huxley & Simmons, 1970). Curtin & Edman (1989) showed that these differential movements are reduced in the fatigued state and this was associated with an increased resistance of the fibre to stretch. Subsequently they confirmed the increased resistance to stretch and showed that muscle stiffness was increased relative to force in the fatigued state and suggest that this may act to stabilise the fibre. Human muscle working *in situ* does not show the two phases of relaxation but it does become more resistant to stretch when fatigued (de Ruyter *et al.* 2000). In a two-state cross bridge model the rate constant for attachment must include a number of steps, from the actual attachment through one or more intermediary steps to the formation of the high force state. Consequently an apparent decrease in the constant f could be as a result of an increased population of cross bridges that are attached but in low force intermediate states. This population would add resistance to stretch but reduce force during shortening and so could stabilise sarcomere lengths and prevent stronger sarcomeres extending weaker ones during relaxation, thereby slowing the decay of force. An accumulation of cross bridges in the low force state would also have the effect of reducing curvature and thus power output of the fatigued muscle. Thus a decrease in the apparent rate of cross bridge attachment could explain both slowing of relaxation and loss of power.

An increase in the concentration of inorganic phosphate is one way in which the proportion of attached cross bridges in the low force state could increase and consequently a clear correlation might be seen between accumulation of P_i and slowing of contractile properties. While the data shown in Fig. 5B do not rule this out,

they are not convincing evidence of a causal relationship. An increase in H^+ might also affect cross bridge kinetics and, again, the data in Fig. 5C are inconclusive. The combination of P_i and H^+ leads to an increase in monobasic phosphate and the data in Fig. 5D do suggest a stronger, possibly causal, relationship with contractile properties. Nevertheless, this cannot be the whole explanation since relaxation becomes slowed in patients with myophosphorylase deficiency in the absence of any increase in H^+ or monobasic P_i (Cady *et al.* 1989b). It is possible that relationships between changes in metabolite levels and contractile function are obscured by compartmentation of metabolites either within or between fibres.

The slowed contractile speed and loss of muscle power seen with fatigue are major limitations to activity in sport and everyday life yet very little is known about the molecular basis of these changes in function. The present results add weight to the suggestion that it is a reduction in the apparent rate of cross bridge attachment leading to an accumulation of cross bridges in low force states that underlies these changes. Attention thus focuses on factors that affect the stages in the cross bridge cycle concerned with attachment and the transition from low to high force states as causes of contractile slowing in fatigued muscles.

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Author contributions

The authors contributed equally to the design of the study, the experimental work, subsequent analysis and drafting of the manuscript. The experimental work was carried out in the Department of Medicine, University College London.