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CHANGES IN FORCE AND INTRACELLULAR METABOLITES DURING FATIGUE OF HUMAN SKELETAL MUSCLE

BY E. B. CADY*, D. A. JONES‡, J. LYNN AND D. J. NEWHAM†

*From the Departments of Medicine, *Medical Physics and †Physiology, University College London, The Rayne Institute, University Street, London WC1E 6JJ*

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SUMMARY

1. The relationship between intracellular metabolites and the generation of force during fatigue has been examined in the first dorsal interosseous muscle of the hand. With the arm made ischaemic, the muscle was fatigued by three bouts of maximal voluntary contraction, leaving approximately three minutes ischaemic rest between contractions. During one series of experiments intracellular phosphorus metabolites were measured by nuclear magnetic resonance during the intervals between the fatiguing contractions; in the second series contractile properties were tested with brief electrical stimulation during the rest intervals.

2. The relationships between loss of force and change in metabolite concentrations obtained with four normal subjects were compared with those from one subject with myophosphorylase deficiency (MPD) who could not utilize muscle glycogen and therefore produced no hydrogen ion from glycolysis during exercise.

3. For both the MPD and normal subjects the relationship between relative force loss and inorganic phosphate (P_i) concentration was curvilinear, force changing little in the early stages of the contraction when the intracellular P_i was accumulating rapidly but falling faster when the P_i was above 25 mM and increasing relatively slowly.

4. In the normal subjects intracellular pH fell from a mean of $7{\cdot}03 \pm 0{\cdot}01$ (mean $\pm$ S.E. of mean, $n = 19$) in the fresh muscle to $6{\cdot}51 \pm 0{\cdot}02$ at the end of the fatiguing exercise; force, as a percentage of the initial value, fell in proportion to the increase in H^+ concentration. In the MPD subject pH did not change and force loss was therefore independent of H^+ accumulation. In the normal subjects the force of the fatiguing muscle showed an approximately linear relationship with the concentration of the monobasic form of inorganic phosphate. However the MPD subject showed a quite different relationship, with force loss being much greater for a given concentration of monobasic phosphate. This result indicates that monobasic phosphate is not a unique determinant of force loss in fatigued muscle.

5. During the first 60 s of recovery in the normal subjects, pH remained low while force recovered, indicating a mechanism of force loss that was independent of H^+ accumulation. However, the recovery of force was not complete, so that for comparable phosphocreatine contents the recovering, more acid, muscle generated

‡ To whom correspondence and reprint requests should be sent.

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less force than the muscle that was being fatigued. It was estimated that H^+-dependent and independent mechanisms contributed roughly equally to the observed force loss. The relationship between force and the concentration of monobasic phosphate differed in fatiguing and recovering muscle.

INTRODUCTION

In the last 20 years there has been continued interest in the relationship between muscle metabolite levels and the generation of force during fatiguing exercise (e.g. Spande & Schottelius, 1970; Edwards, Hill & Jones, 1975; Dawson, Gadian & Wilkie, 1978; Hultman & Sjöholm, 1983). There are a number of metabolic changes that occur concurrently with fatigue but none has been shown to account fully for the extent or time course of force loss in intact muscle preparations. It has long been known that ATP remains relatively constant at times when force is markedly reduced (Spande & Schottelius, 1970; Edwards *et al.* 1975; Hultman & Sjöholm, 1983) and this has been confirmed by nuclear magnetic resonance (NMR) studies which overcome the uncertainties associated with direct chemical analysis (Dawson, Gadian & Wilkie, 1977, 1978). More recently attention has turned to H^+ and the products of the ATPase reaction, ADP and inorganic phosphate (P_i) which could, acting separately or in combination, inhibit force generation.

In skinned skeletal and cardiac muscle preparations inorganic phosphate has been shown to have a marked effect on force (Cooke & Pate, 1985; Kentish, 1986; Nosek, Fender & Godt, 1987; Cooke, Franks, Luciani & Pate, 1988; Chase & Kushmerick, 1988), with the greatest sensitivity being in the range of concentrations up to about 10 mM. With intact preparations, however, the main rise in P_i occurs in the early part of the contraction when there is relatively little loss of force. The pK of the reaction $HPO_4^{2-} + H^+ \rightleftharpoons H_2PO_4^-$ is close to 7 so that the proportions of the mono- and dibasic forms of phosphate will vary greatly with change of pH in the physiological range. The appearance of the monobasic species of inorganic phosphate ($H_2PO_4^-$) in the fibre will lag behind that of the total phosphate and more closely follow the change in force, suggesting that this may be the cause of fatigue (Dawson, Smith & Wilkie, 1986; Wilkie, 1986; Nosek *et al.* 1987; Miller, Boska, Moussavi, Carson & Weiner, 1988).

We have examined the relationship between intracellular phosphorus metabolites, H^+ and force during fatiguing contractions of the human first dorsal interosseous muscle of the hand. Subjects with normal muscles have been studied together with one subject who suffered from myophosphorylase deficiency (MPD) and consequently produced no H^+ from muscle glycolysis during exercise.

METHODS

Subjects

Four normal subjects (two male, two female, aged 21–43 years) were studied; these were three of the investigators and one colleague. The subject with MPD was female, 65 years of age, and had previously been diagnosed on the basis of clinical symptoms and the absence of the enzyme in a muscle biopsy specimen from the quadriceps muscle. She had previously been the subject of similar physiological investigations and, after the purpose and procedures had been fully explained, agreed

to help with the present series of experiments. The procedures used for fatiguing and stimulating muscle and for the nuclear magnetic resonance measurements were approved by the local Ethical Committee.

Experimental design

The first dorsal interosseous muscle was fatigued in a stepwise fashion by three maximal voluntary contractions (MVC). Between each fatiguing MVC there was a rest of approximately 3 min during which the muscle was tested. Throughout the fatiguing and testing procedures the muscle was maintained ischaemic by a cuff inflated around the upper arm. In the first set of experiments muscle metabolites were measured by NMR, and in parallel studies conducted outside the magnet, the muscle contractile properties were measured by imposing brief electrical stimulation during the rest intervals. For the normal subjects the three fatiguing contractions were each of 15 s duration, giving a total of 45 s MVC. The MPD subject exercised and was tested in the same way except the three fatiguing MVCs were of 7 s duration. The duration was restricted in this way to minimise the risk of the MPD subject developing a muscle contracture. After the third period of ischaemic rest the circulation was restored and muscle function and metabolites were followed during recovery for up to 10 min.

In a second series of experiments the protocol was changed to allow comparisons to be made between the onset and recovery from fatigue; full details are given in the following paper (Cady, Elshove, Jones & Moll, 1989). There was a 3 min period of ischaemia with the muscle at rest before the first fatiguing contraction. During the fatiguing phase the subjects began with two short 7 s contractions followed by a 30 s contraction to deplete the phosphocreatine (PCr) stores. Recovery was then allowed to proceed in steps. The circulation was restored for 30 s and then occluded for 3 min while the muscle was tested. The circulation was then restored for another 30 s before the circulation was again occluded while measurements were made of muscle force and metabolites. In this series of investigations three normal subjects undertook two runs each for metabolite measurements and between four and seven (total of sixteen) runs for measurements of contractile properties.

Hands were warmed for 10 min in hot water to ensure that the muscles were close to core temperature at the start of the experiment.

Changes of contractile properties

In experiments conducted on three subjects outside the magnet, the pronated hand was immobilized with the thumb abducted. Isometric force of the first dorsal interosseous was measured with a strain gauge applied to the side of the first finger at the first interphalangeal joint (Jones, Rutherford & Whiteson, 1987). For the fatiguing exercise the subjects performed MVCs with visual feedback of the force signal and verbal encouragement while, for testing, the muscle was stimulated for 0·5 s at 100 Hz by supramaximal square-wave pulses applied to the ulnar nerve at the wrist or elbow. Three baseline measurements were made at 1 min intervals prior to the cuff being inflated around the arm. During the ischaemic rest periods, either between the fatiguing contractions or after 30 and 60 s recovery, the muscles were tested three times with electrical stimulation at 1 min intervals. Only the results for the stimulated contractions are reported as these are objective measures of muscle function not subject to problems such as loss of central drive or extraneous movements of the forearm, as may be the case with voluntary contractions.

Changes in muscle metabolites

For the parallel experiments conducted inside the magnet of the NMR spectrometer, the hand was held in the apparatus described by Aldridge, Cady & Newham (1987), which has a strain gauge mounted in the same position as for experiments outside the magnet so that the fatiguing voluntary contractions involved the same movement in the two sets of experiments. There was visual feedback of the force signal and verbal encouragement to ensure that the efforts were truly maximal. Repeated measurements were made of four subjects.

Relative concentrations of muscle metabolites were measured by NMR spectroscopy using an Oxford Research Systems – Bruker spectrometer equipped with a 1·9 tesla, 26 cm clear-bore superconducting magnet. A two-turn surface coil, operating in the receive/transmit mode with switchable tuning for both ^{31}P (32·5 MHz) and ^{1}H (80·3 MHz), was placed directly over the first dorsal interosseous. Radio frequency pulses giving a 90 deg flip angle at the coil centre were used,

with a relaxation interval of 2·256 s. A complete run consisted of a resting spectrum (96 scans), a spectrum from the ischaemic resting muscle following each fatiguing MVC (96 scans each) and a number of collections during recovery after the circulation was restored (32 scans each). The resultant free induction decays were processed with zero filling (1024–2048 points), 16 Hz line broadening and baseline flattening (Akitt, 1978; Gordon, Hanley & Shaw, 1982) before Fourier transformation. After phasing and further baseline flattening procedures involving the spectrum integral, the areas of the resonances were measured by setting cursor positions on either side of each peak and integrating under the line profile between them. Peak positions were determined by finding the highest point between the cursors and calculating a weighted mean using the surrounding seven points. Areas were measured for the phospho-monoesters (including sugar phosphates; PM), P_i, phospho-diesters (PD), PCr and the α- and β-ATP resonances. The peak areas were then corrected for partial saturation using the following factors: PM, 1·19; P_i, 1·22; PD, 1·11; PCr, 1·47 and β-ATP, 1·21. The total mobile (and hence NMR visible) phosphorus was taken to be the sum of $P_i + PCr + PM + PD + 3 \times \beta$-ATP, and the individual compounds were expressed as a fraction of the total. In nineteen runs with the four normal subjects the ATP as a fraction of the total mobile phosphate in resting muscle was $0{\cdot}101 \pm 0{\cdot}017$ (mean ± S.D.). Intracellular concentrations were estimated as described by Dawson *et al.* (1978), Dawson (1982) and Taylor, Styles, Matthews, Arnold, Gadian, Bore & Radda (1986), making a number of assumptions about muscle ATP and water content. If the ATP concentration is 5·5 mmol kg^{-1} fresh muscle and the intracellular water content is 67 %, the intracellular ATP concentration is 8·2 mM. Combining this concentration with the value for the fractional ATP content, the total mobile phosphate becomes 81·4 mM. This value was used to calculate the absolute cytosolic concentrations of the various metabolites. The fractional ATP content for the MPD subject was $0{\cdot}106 \pm 0{\cdot}020$ (mean ± S.D. of four measurements of resting muscle). This was not significantly different from the value for the normal subjects, and consequently the same values have been used to estimate the intracellular concentrations of phosphorus metabolites.

Intracellular pH was estimated from the chemical shift of the P_i peak with respect to PCr (δ) using the following form of the Henderson–Hasselbach equation (Dawson *et al.* 1977; and D. R. Wilkie, personal communication):

$$pH = 6{\cdot}73 + \log_{10}[(\delta - 3{\cdot}275)/(5{\cdot}685 - \delta)].$$

The free intracellular ADP concentration was calculated from the estimated concentrations of ATP, PCr, H^+ assuming that the total creatine and phosphocreatine remained constant throughout exercise at 42·5 mM:

$$[ADP] = ([42{\cdot}5 - PCr][ATP])/([PCr][H^+]K_{eq}).$$

The equilibrium constant (K_{eq}) for the reaction was taken to be $1{\cdot}66 \times 10^9$ M^{-1}.

The concentration of the monobasic phosphate was estimated from the total P_i and pH:

$$[H_2PO_4^-] = ([H^+][P_i])/(K_{P_i} + [H^+]).$$

The equilibrium constant K_{P_i} was taken to be $1{\cdot}86 \times 10^7$ M^{-1}.

RESULTS

Muscle force

The MVC and stimulated force of the fresh muscles varied twofold between the normal subjects, the stimulated forces ranging from 40 to 80N. To combine data from different subjects and occasions the values have been expressed as a percentage of the force recorded for the fresh muscle prior to ischaemia and exercise.

Figure 1 shows that during the 3 min rest period after the first and second fatiguing contraction there was a tendency for the tetanic force to decline but this did not reach statistical significance. In the third period, however, the first and third

measurements were significantly different (paired t test, $P < 0{\cdot}01$), with the force decreasing by about 10%. Force recovered to at least 90% of the resting value by 5 min recovery with a restored circulation.

The MPD subject experienced no difficulties with the experimental procedures

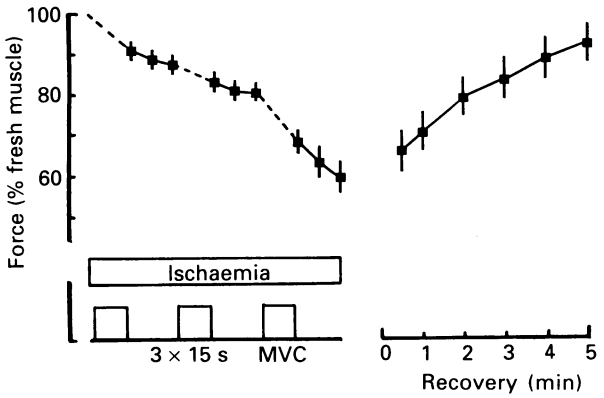

Fig. 1. Tetanic force during the fatiguing protocol and subsequent recovery in three normal subjects (16 runs, mean ± S.E. of mean). The arm was made ischaemic and the subjects performed three 15 s MVCs, with 3 min rest periods between during which time the muscle was tetanically stimulated at 100 Hz for 0·5 s every minute.

and, as with the normal subjects, the ulnar nerve stimulation remained supramaximal throughout. The stimulated force generated was 50 N and both this and the MVC were comparable with forces produced by the normal female subjects. The MPD subject showed the same tendency for force loss during the 3 min rest intervals and recovered to 96% of the fresh resting value within 5 min of restoring the circulation.

Muscle metabolite measurements were the average of scans taken over the entire 3 min interval between fatiguing contractions; therefore, to compare changes in force and metabolites the mean of the three measurements of force has been taken. Results for the individual subjects are given in Table 1, indicating the variation between normal subjects and showing clearly that the MPD subject fatigued more than any of the normal subjects. The data in Table 1 from sixteen runs on three normal subjects have been combined and Fig. 2 shows a comparison of the mean tetanic force, as a percentage of the fresh muscle force, during the intervals following the fatiguing activity as a function of the duration of the fatiguing MVC. Normal subjects declined to 64% ± 2·3 (mean ± S.E. of mean) of the control value after 45 s MVC. The MPD subject showed a greater loss of tetanic force which declined to 49% ± 5 after only 21 s of MVC.

Muscle metabolites

Figure 3 shows typical spectra from a normal subject and the MPD subject at rest, during the fatiguing activity and subsequent recovery. At least four measurements were made on each person on different occasions and the mean values for the individual normal and MPD subjects are given in Table 1. The values are very similar

to those that can be calculated from reports of human gastrocnemius muscle, wrist flexors and first dorsal interosseous (Griffiths & Edwards, 1984; Aldridge, Cady, Jones & Obletter, 1986; Taylor *et al.* 1986; Aldridge *et al.* 1987). The fresh values for the MPD subject were similar to those of the normal subjects except that there was a tendency for the P_i to be slightly raised and PCr to be slightly lower. Metabolite measurements made during the fatiguing exercise are also given in Table 1 but in the figures all nineteen runs on the normal subjects have been combined.

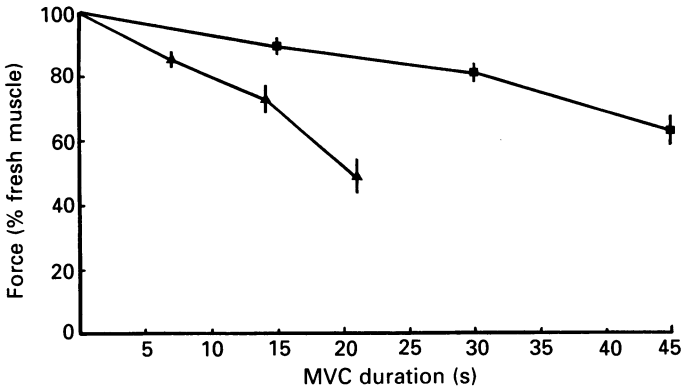

Fig. 2. Changes in tetanic force during the fatiguing exercise in normal subjects (■, $n = 16$) and the MPD subject (▲, $n = 4$). Force (mean ± S.E. of mean) is expressed as a percentage of the fresh value and plotted as a function of the cumulative fatiguing MVC duration.

As a result of the three 15 s contractions, PCr in the normal subjects fell to about one-sixth of the resting value (38·3 ± 0·4 to 6·3 ± 0·5 mM; mean ± S.E. of mean) with a nearly equimolar rise in P_i (6·1 ± 0·3 to 32·9 ± 1·0 mM). The concentration of ATP remained constant throughout the experimental period and pH fell by approximately half a unit (7·03 ± 0·01 to 6·51 ± 0·02; Fig. 4*A*). For the MPD subject the three 7 s contractions resulted in very similar changes in muscle metabolites (Fig. 4*B*), with the notable exception that pH did not change as a result of the fatiguing activity; indeed there was a tendency for pH to increase (7·06 ± 0·02 to 7·11 ± 0·03) but this did not achieve statistical significance.

Muscle metabolites returned to resting levels by about 5 min after restoring circulation in both normal and MPD subjects.

Force and muscle metabolites

Changes in the relative tetanic force production were clearly not related to changes in free ATP as measured in these experiments (compare Figs 1 and 4). Figure 5*A* shows the relationship between relative force and total inorganic phosphate. For a similar extent of PCr breakdown and total P_i accumulation, the MPD subject showed a greater percentage loss of force compared to the normal subjects. In both normal and MPD subjects there was a curvilinear relationship between force and P_i, the major change in P_i occurring during the first two fatiguing contractions and before force began to decline to a marked extent.

The concentration of the monobasic form of phosphate ($H_2PO_4^-$) was calculated

from the total P_i and pH. In the normal subjects approximately one-third of the phosphate was in the monobasic form in the fresh muscle with a pH of 7. In the fatigued muscle, with a pH of 6·5, about two-thirds was in the monobasic form; Fig. 5*B* shows the relationship between the change in relative force and monobasic

TABLE 1. Intracellular metabolite concentrations, pH and force in fresh resting muscle and during fatiguing exercise.

Subject		pH	P_i (mM)	PCr (mM)	ATP (mM)	Force (%)
N1	C	7·08±0·02	7·1±0·3	38·7±0·7	7·9±0·9	100
	1	6·83±0·02	20·2±0·8	21·4±1·9	7·9±0·9	91·4±2·8
	2	6·70±0·01	29·4±0·8	12·0±0·4	7·5±0·6	84·2±3·6
	3	6·53±0·03	33·0±1·5	8·6±0·5	7·9±0·5	56·4±3·5
N2	C	7·02±0·00	5·9±0·5	39·6±1·3	8·3±0·6	100
	1	6·83±0·01	22·1±0·7	18·4±1·5	8·5±0·6	90·6±1·7
	2	6·59±0·02	30·9±0·8	9·1±1·0	7·8±0·4	82·4±1·8
	3	6·46±0·01	37·2±0·7	4·0±0·5	7·8±0·4	64·0±4·0
N3	C	7·01±0·02	6·6±0·9	38·1±0·7	7·4±0·9	100
	1	6·86±0·02	21·8±1·4	18·9±0·6	8·6±0·5	85·6±0·8
	2	6·66±0·03	28·8±0·6	9·3±0·6	7·8±0·4	78·5±1·2
	3	6·57±0·07	34·1±1·6	5·8±1·6	7·5±0·8	69·2±2·5
N4	C	7·02±0·03	5·5±0·4	37·0±0·2	8·9±0·3	
	1	6·83±0·03	19·5±0·4	20·4±0·7	9·0±0·3	
	2	6·67±0·03	25·6±0·6	9·3±0·3	10·5±0·2	
	3	6·49±0·03	28·4±1·1	7·0±0·6	9·8±0·3	
All	C	7·03±0·01	6·1±0·3	38·3±0·4	8·2±0·3	100
	1	6·83±0·01	20·8±0·5	19·6±0·6	8·6±0·3	89·1±1·3
	2	6·65±0·01	28·4±0·6	9·8±0·4	8·6±0·4	81·6±1·4
	3	6·51±0·02	32·9±1·0	6·3±0·5	8·4±0·3	63·7±2·5
MPD	C	7·06±0·01	7·6±0·7	36·8±1·1	8·6±0·7	100
	1	7·10±0·01	15·9±0·9	27·1±1·0	8·0±0·3	85·4±1·5
	2	7·11±0·02	23·1±1·1	19·6±0·4	7·8±0·8	73·7±3·5
	3	7·11±0·02	27·4±1·5	12·7±1·0	8·3±0·6	48·8±5·5

Data are for four normal subjects (N1–4) and one MPD subject and are given as the mean ± S.E. of mean from between four and six runs on each individual. Values were from control resting muscle (C) and during the rest interval following each of three fatiguing contractions (1–3). Values for force are expressed as a percentage of the force of the fresh muscle. In the rows labelled 'All' are given the mean values for all nineteen runs on normal subjects. Measurements of force were made on only three of the normal subjects (total of sixteen runs).

phosphate. In the normal subjects, force declined roughly in proportion to the increase in monobasic phosphate. The MPD subject showed a quite different relationship, with a greater loss of force for an equivalent increase in monobasic phosphate.

Figure 5*C* shows the change in tetanic force as a function of intracellular H^+ concentration. For the normal subjects, force declined roughly in proportion to the increase in H^+ but the results for the MPD subject were quite different, showing that in this case force loss was not dependent on H^+ accumulation. Estimates of free ADP concentration are subject to considerable errors since the calculation compounds the errors involved in estimating ATP, PCr and pH. However, plotting the relative force

during fatigue as a function of ADP concentration (Fig. 5*D*) suggests there might be a linear relationship which was similar in form for the normal and MPD subjects.

It was noticed that after restoration of the circulation pH in the normal subjects remained low, around 6·5, for at least 2 min (see following paper, Cady *et al.* 1989).

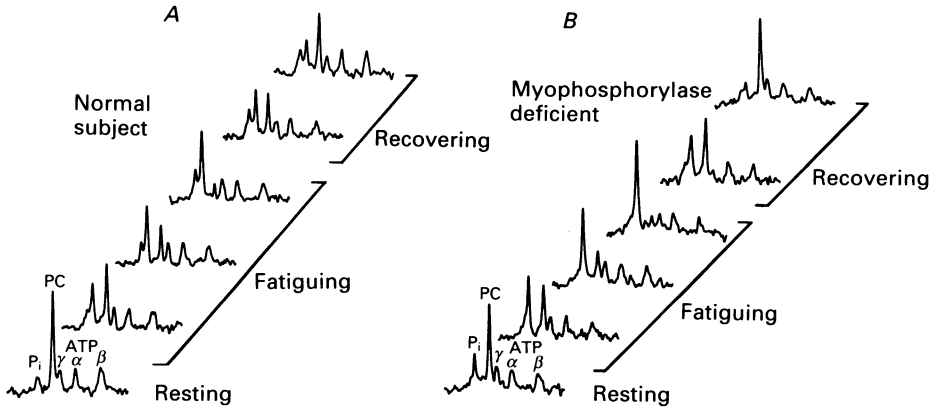

Fig. 3. NMR spectra from: *A*, normal subjects; *B*, MPD subject, before, during and after the fatiguing exercise.

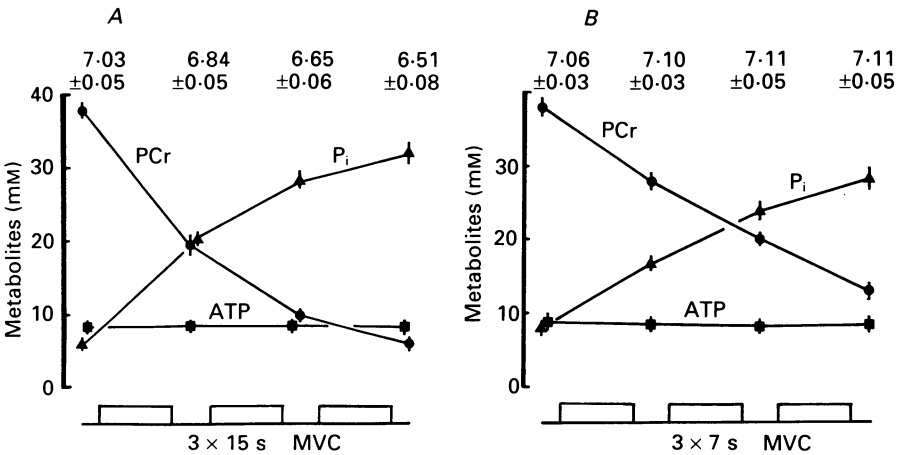

Fig. 4. Changes in muscle metabolites and pH during fatiguing exercise. *A*, normal subjects. *B*, MPD subject. Measurements are shown for the fresh muscle and the values obtained during the intervals between fatiguing contractions, corresponding to the times when force measurements were made as shown in Fig. 1. The values given above the Figures are the estimated intracellular pH. Values are the mean ± S.E. of mean of nineteen runs (normal subjects) or four runs for the MPD subject.

while the pH of the MPD muscle was approximately 7·1 during this time. In the 2 min recovery period force of the normal subjects returned to 85 % ± 2·4 (mean ± S.E. of mean) of the value for the fresh muscle. With the MPD subject, force recovered to a greater extent (95 % ± 2·2), raising the possibility that the continued acidosis in

the normal subjects may have depressed force recovery. The experimental protocol was therefore altered to allow a better comparison of force and metabolite levels during recovery. In the second series of experiments on normal subjects, muscles were fatigued and allowed to recover in a stepwise fashion. The extent of metabolite

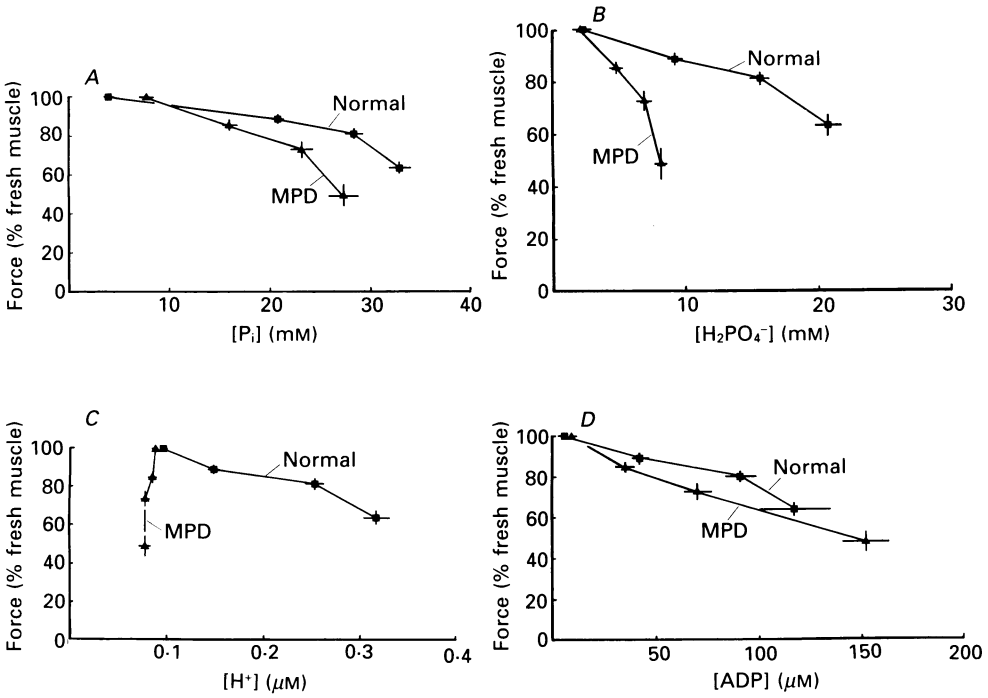

Fig. 5. Tetanic force as a function of intracellular metabolite concentrations during the fatiguing exercise for normal (■) and MPD subjects (▲). Values are the mean ± S.E. of mean for nineteen runs for metabolites and sixteen runs for force (normal subjects) or four runs of each for the MPD subject. *A*, inorganic phosphate and force. *B*, monobasic phosphate and force. *C*, hydrogen ion concentration and force. *D*, ADP and force.

depletion was expressed as change in PCr compared with resting concentrations. Force generated as a function of PCr depletion is given in Fig. 6*A* and shows a different relationship for fatiguing muscles compared with muscles that were recovering. Comparing muscles with a similar degree of PCr depletion, the recovering muscle generated a lower force than those that were fatiguing. In the recovering muscles pH was approximately 0·35 units lower than in the fatiguing muscles. With the recovering muscles there was a significant increase in force as PCr content increased, despite the fact that pH remained low for the first 60 s after the circulation was restored.

A consequence of the continuing acidosis during recovery was that the proportion of the total phosphate present in the monobasic form was greater than in the fatiguing muscles for a similar total P_i content. Figure 6*B* shows the relationship between force and monobasic phosphate for the second series of experiments for muscles that were fatiguing and recovering. The relationship in the fatiguing muscles

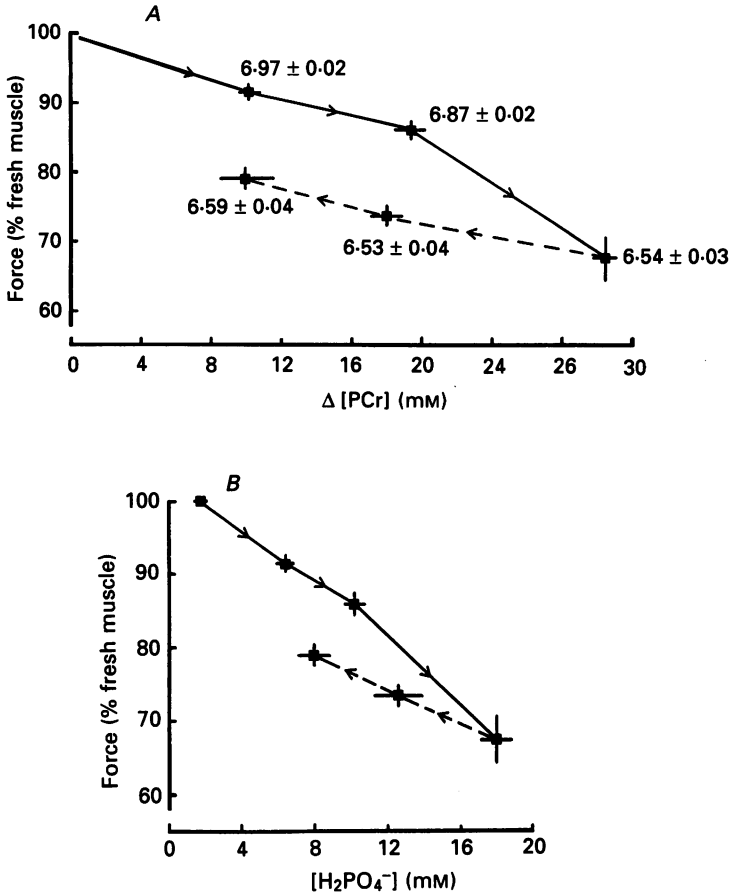

Fig. 6. The relationship between metabolite changes and generation of force in normal subjects during fatigue and recovery. Data are from the second series of experiments where the muscles were fatigued with two 7 s MVCs followed by a 30 s MVC. The data show the change in force generated in relation to the metabolite change during the fatiguing phase of the protocol (points connected by a continuous line) and after 30 and 60 s recovery (points connected by a dashed line). The arrows indicate the sequence of the observations. Data are given as mean ± S.E. of mean for six runs for metabolites and sixteen runs for force. *A*, force and PCr depletion. Depletion (△) refers to the change relative to the resting PCr concentration. Numbers on the figure indicate the pH (mean ± S.E. of mean) at the time when the PCr measurements were made. *B*, force and monobasic phosphate concentration.

was similar to that seen in the first series (Fig. 5*B*) but this was not the case during recovery, demonstrating that there is no unique relationship between monobasic phosphate and force production.

DISCUSSION

The metabolic basis of force fatigue in skeletal muscle has long been a subject of study, with much speculation about the role of H^+ and other metabolite

accumulation in the exercising muscle. The present results show that force generation in intact muscle is not as sensitive to the accumulation of inorganic phosphate as in skinned fibre preparations, and that accumulation of monobasic phosphate and H^+ are not the only causes of fatigue during high-force isometric exercise lasting up to 45 s.

The use of human muscle, working *in situ*, has a number of advantages. In healthy subjects it is reasonable to assume that the muscle is in good condition and, if sufficient time is left between runs, it is possible to make repeated observations on the same muscle. The most important advantage is that recovery can be followed to check that the changes are reversible and do not involve processes that go beyond the usual understanding of the word 'fatigue' to include long-lasting damage or muscle fibre death. In the present experiments muscle force and metabolites had fully recovered within 10 min of the end of exercise in both normal and MPD subjects. The observation of complete recovery in the latter subject is important since her specific enzyme deficiency can result in painful and damaging contractures which develop after heavy exercise and only resolve slowly.

Inevitably, however, there are difficulties and uncertainties associated with the techniques and experimental design we have used. The major concern is that comparisons have been made between two separate sets of experiments, one involving measurements of muscle metabolites by NMR and the other the assessment of changes in force generation. The validity of this comparison rests on whether the fatiguing contractions had the same effects on muscle metabolites and force generation in the two series of experiments. Maximum voluntary abduction of the index finger is a difficult movement to make in a reproducible fashion and the measured forces may involve other muscles in the hand or movements of the forearm. For this reason the subjects were asked to make maximal contractions in which it was likely that the first dorsal interosseous would be fully activated (if together with other muscles) rather than given a submaximal target which might be achieved using an uncertain and variable portion of the first dorsal interosseous. The relatively small variance of the force measurements shown in Fig. 1 suggests that the voluntary fatiguing activity changed the contractile properties of the muscle in a consistent manner. One further problem arises from the statistical treatment of the data. To facilitate comparisons of the relationships between force and muscle metabolites in normal and MPD subjects, all the runs on normal subjects have been combined. Since there were several runs on each individual the values cannot be regarded as strictly independent and therefore the standard errors quoted are somewhat smaller than they might be if the observations were truly independent. However, none of the conclusions to be drawn from these data depend on fine statistical analysis, the crucial observations being that in the MPD subject there was less H^+ accumulation than in the normal subjects; secondly that there were similar changes in phosphorus metabolites, and lastly that the degree of fatigue was at least as great in the MPD subject as in the normals. That these three facts were true can be clearly seen from the data presented in Table 1 without the benefit of statistical analysis.

The shape of the relationship between loss of force and P_i for both normal and MPD subjects was similar to that reported for other intact skeletal muscle preparations in which P_i has been measured either chemically (Sahlin, Edström,

Sjöholm & Hultman, 1981) or by NMR (Dawson *et al.* 1978). The form of the relationship is also similar to estimates that can be made of P_i from changes of PCr in a variety of other studies (e.g. Edwards *et al.* 1975; Hultman & Sjöholm, 1983) if an allowance is made for PCr hydrolysis that would have occurred during freezing of the samples. A comparison of the present results with the relationships between force and P_i reported for skinned fibre preparations shows that intact preparations are much less sensitive to P_i. The present results (Fig. 5*A*), and those of Dawson *et al.* (1978) and Sahlin *et al.* (1981) with intact preparations, show that as high a concentration as 20 mM-P_i is associated with a relatively modest loss of force of around 10–20% and this in the presence of some acidification. In contrast, Cooke & Pate (1985) reported that, for skinned fibre bundles, 20 mM-P_i resulted in about 30% loss of force at pH 7·0 and 70% at pH 6·5; their data also indicate that the maximum effect of P_i is seen by around 10 mM. Nosek *et al.* (1987) reported a 25% reduction at 20 mM, with data that also suggests most of the effect is obtained at around 10 mM and is greater under acid conditions. Chase & Kushmerick (1988) report that 15 mM-P_i resulted in a 30% reduction of force at pH 7·0 and 50% at pH 6·5. Skinned cardiac preparations also appear to be very sensitive to P_i (Kentish, 1986).

Not only is there a quantitative difference in the effects of P_i on intact and skinned preparations, but there is a qualitative difference in the form of the relationship. Skinned preparations show greatest sensitivity at low P_i concentrations, with a maximum effect at about 10 mM, giving a concave relationship; the intact preparations, in contrast, show a convex relationship (Fig. 5*A*). The resting P_i concentration of the human muscles (Table 1) was found to be relatively high when compared to the composition of buffers used to obtain maximum force in skinned fibre preparations. It is possible, therefore, that the force of the fresh human muscle is less than might be obtained were skinned preparations to be made and incubated with low concentrations of inorganic phosphate. However, this seems unlikely as estimates of the maximum force generated per unit cross sectional area are very similar for intact and skinned preparations (Elzinga, Stienen & Wilson, 1988).

One explanation for the curvilinear relationship between P_i and force in intact muscle preparations is that the increase in P_i is associated with a decrease in pH, and that the monobasic form, $H_2PO_4^-$, is responsible for the loss of force (Dawson *et al.* 1986; Wilkie, 1986). The results shown in Fig. 5*B* for the normal subjects indicate that the relationship is indeed more linear than that for force and total P_i (Fig. 5*A*), although the slope of the relationship in Fig. 5*B* is less steep than that reported by Wilkie (1986) or Miller *et al.* (1988) for intact preparations or Nosek *et al.* (1987) for skinned muscle fibres. For the MPD subject, pH did not change during the fatiguing process and the proportion of P_i in the $H_2PO_4^-$ form remained constant at about one-third of the total. The relationship between force and $H_2PO_4^-$ was therefore quite different for normal and MPD subjects (Fig. 5*B*). Although Sahlin *et al.* (1981) did not consider the role of $H_2PO_4^-$ their results, concerning metabolite levels and force in muscles poisoned with iodoacetate, are similar to those presented here. They found a somewhat greater loss of force in the poisoned muscles for a given rise in P_i when the $H_2PO_4^-$ would have been lower than in the unpoisoned muscle.

The concentration of ADP cannot be directly measured from the NMR spectra but the estimates shown in Fig. 5*D* suggest that there may be a relationship between

force loss and free ADP accumulation that is similar for both normal and MPD subjects. It is not entirely clear how an accumulation of ADP would affect force generation by the actomyosin cross-bridges, since Cook & Pate (1985) found that concentrations in excess of the normal physiological range (up to 2 mM) caused an increase in force in the skinned preparations rather than the decrease seen in fatigued intact muscle.

Hydrogen ion is widely reported to depress force production in both skinned (Donaldson & Hermansen, 1978; Cooke *et al*, 1988; Nosek *et al*. 1987; Chase & Kushmerick, 1988) and intact preparations (Edman & Mattiazzi, 1981). In the present study the loss of force was well matched by the increase in H^+ concentration (Fig. 5*C*) for the normal subjects, and this was similar to the findings of Dawson *et al*. (1978) and Sahlin *et al*. (1981) with unpoisoned muscle. The results for the MPD subject, however, show a quite different relationship, with force loss being independent of H^+ accumulation. This observation is similar to that made by Sahlin *et al*. (1981) for poisoned muscles, and is not unexpected in the light of previous work with poisoned mouse muscle (Edwards *et al*. 1975) and subjects with MPD and phosphofrucktokinase deficiency (Wiles, Jones & Edwards, 1981). In the MPD muscle there was clearly a mechanism causing a loss of force that was independent of H^+ accumulation. Such a mechanism also exists in normal muscle, as can be seen in the results for normal subjects illustrated in Fig. 6*A*. When the blood supply was restored to the fatigued muscle, PCr was resynthesized and force recovered despite the continued low pH during the first 60 s. There is also evidence from these data that in normal muscle there is, in addition, a H^+ dependent mechanism of force fatigue. During recovery the force generated for a given level of intracellular PCr was less when the intracellular pH was low (Fig. 6*A*).

The existence in normal muscle of two mechanisms leading to a loss of force, as opposed to the single mechanism in MPD muscle, might be expected to result in a more rapid fatigue in normal subjects. In fact the reverse was found to be the case. For similar levels of metabolite depletion (as judged by P_i accumulation, Fig. 5*A*) the MPD muscle lost more force than did the normal muscle. An explanation for this might be a premature loss of membrane excitability which would give rise to force loss without metabolite change. Early failure of action potential amplitude has been reported in MPD patients (Wiles *et al*. 1981) but it is not certain that this is the explanation for the greater loss of force in relation to metabolic change. In Fig. 4*B* it can be seen that PCr depletion continues at a fairly uniform rate throughout the exercise, giving no indication of a sudden failure of excitation in the MPD subject.

The quantitative significance of the two mechanisms of force fatigue in normal muscle is difficult to judge. The results in Fig. 6*A* indicate that a change of about 20 mM in the concentration of PCr after 60 s recovery, when pH remained low and relatively constant, was associated with a 10% increase in force. The force generated by fatiguing and recovering muscles with a similar PCr concentration (e.g. first and last points in Fig. 6*A*), was depressed by about 10% for a decrease in pH of about 0·35 units. It appears, therefore, that the effects of H^+ accumulation and depletion of phosphorus metabolites were having roughly similar effects on the generation of force.

An inhibitory effect of monobasic phosphate on force production would provide a

unifying mechanism combining the pH- and phosphorus metabolite-dependent causes of fatigue. However, the results for the MPD subject (Fig. 5*B*) indicate that, as with H^+, monobasic phosphate accumulation cannot be the sole explanation for the loss of force. The results illustrated in Fig. 6*B* show that for normal muscle the observed relationship between monobasic phosphate and force seen during the development of fatigue does not apply during recovery, and suggest that the relationship seen here and reported by others for fatiguing muscle (Dawson *et al.* 1986; Miller *et al.* 1988) may be largely coincidental rather than causal.

Whatever the nature of the biochemical change underlying fatigue there is also the question of the site of action, whether at the level of cross-bridge interaction or the processes of excitation–contraction coupling. In certain situations, especially where muscle is fatigued by stimulation at high frequencies, there is evidence that the contractile machinery continues to function relatively normally, while loss of force is due mainly to changes in membrane excitability (Jones, Bigland-Ritchie & Edwards, 1979; Jones, 1979). Other experiments have shown that caffeine contractures are unaffected when stimulated force is reduced (Nassar-Gentina, Passonneau & Rapoport, 1981; Länngergren & Westerblad, 1989; Jones & Sacco, 1989). It may be useful, therefore, to investigate further the role of intracellular metabolites in controlling membrane excitability and the release of intracellular calcium during fatiguing exercise.

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