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DISSOCIATION BETWEEN METABOLIC AND CONTRACTILE RESPONSES DURING INTERMITTENT ISOMETRIC EXERCISE IN MAN

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SUMMARY

This study examines the temporal changes in high-energy phosphate and metabolite levels, and in force-generating capacity, during and after voluntary submaximal repetitive isometric exercise (RIE). Eight male subjects performed one-legged RIE with the knee extensors at 40 % maximum voluntary contraction (MVC) target force (duty cycle: 6 s contraction, 4 s rest) in a 48 cm bore whole body 1·5 T superconducting magnet. Phosphocreatine (PCr), inorganic phosphate (P_i), ATP and pH were measured every 9 s. Force-generating capacity was repeatedly measured using MVC force and electrically stimulated contractions (sequential train of impulses of 1–100 Hz). During RIE, MVC declined gradually by 56 ± 5 % (mean ± S.E.M.). Electrically stimulated force also declined, with a disproportionally large drop in low-frequency force, seen as a decline from 0·76 ± 0·02 to 0·33 ± 0·02 in 20:50 Hz force ratio. The PCr decline during RIE was 65 ± 9 %, in most subjects seen as a rapid initial drop followed by less or no further decline to exhaustion. pH declined in parallel by 0·18 ± 0·04 units, whilst ATP levels remained unchanged throughout the exercise. PCr, P_i and pH recovered to near control values within 5 min of exhaustion. Force, however, was not fully restored after 30 min recovery. The results support the hypothesis that fatigue from submaximal RIE is unrelated to changes in P_i and H^+ levels. The decline in 20:50 Hz force ratio implies that fatigue may be associated with excitation–contraction coupling impairment. No sudden changes were observed in mechanical or metabolic factors at exhaustion. Exhaustion was probably not caused by lack of substrates for ATP resynthesis, since pH had decreased only marginally.

INTRODUCTION

Fatigue during isometric contraction in human muscle has generally been associated with ischaemia, leading to rapid phosphocreatine (PCr) depletion and accumulation of metabolites (Ahlborg, Bergström, Ekelund, Guarnieri, Harris, Hultman & Nordesjö, 1972; Edwards, Harris, Hultman & Nordesjö, 1972*a*; Sahlin, Harris & Hultman, 1975; Harris, Sahlin & Hultman, 1977). Within this protocol, fatigue, defined as a decline in maximal force (Bigland-Ritchie, Furbush & Woods, 1986*b*; Vøllestad, Sejersted, Bahr, Woods & Bigland-Ritchie, 1988) is explained at the cellular level, where the inhibiting effect of high inorganic phosphate (P_i) and H^+ levels on maximal Ca^{2+}-activated force is well documented (reviewed by Westerblad, Lee, Lännergren & Allen, 1991; Fitts, 1994). However, fatigue from isometric contraction may develop to a similar extent in the absence of such substrate and metabolite changes. During the first 30 min of voluntary repetitive intermittent knee extensor exercise

(RIE) at 30 % MVC, Vøllestad *et al.* (1988) demonstrated a gradual decline in maximal force by ~50 %, whilst PCr, P_i and H^+ levels remained nearly unchanged. Similar experiments suggest that neither central nervous factors nor neuromuscular transmission failure can explain fatigue during submaximal knee extensor RIE (Bigland-Ritchie *et al.* 1986*b*), indicating that peripheral mechanisms are involved. It has been suggested that fatigue from RIE may be associated with an impairment of the excitation–contraction (E–C) coupling, independent of substrate and metabolite changes (Vøllestad *et al.* 1988). More recently Sahlin, Cizinsky, Warholm & Högberg (1992) demonstrated a gradual decline in both MVC and PCr levels in the vastus lateralis muscle from the onset of 30 % MVC RIE, and the metabolic responses to this type of exercise are therefore inconclusive. One aim of the present paper was to re-investigate the temporal pattern of metabolic responses, and how they relate to the changes in maximal force during and after submaximal RIE.

Exhaustion from voluntary isometric contraction is usually defined as the point where maximal force or target force can no longer be upheld. Due to the restriction of blood flow during isometric contraction (Edwards, Hill, Jones & Merton. 1972*b*; Sejersted, Hargens, Kardel, Blom, Jensen & Hermansen, 1984; Saugen & Vøllestad, 1995*b*), exhaustion from sustained activity is associated with high levels of P_i and H^+ ions (Ahlborg *et al.* 1972; Edwards *et al.* 1972*a*; Sahlin *et al.* 1975; Harris *et al.* 1977), and possibly also with a decline in the free energy of ATP hydrolysis (Dawson, Gadian & Wilkie, 1980). During RIE the vastus lateralis muscle is rapidly reperfused between contractions (Vøllestad, Wesche & Sejersted, 1990). Still, a rapid decline in glycogen and PCr levels, together with a small but significant decline in ATP, has been reported during the last 3–4 min before exhaustion (Vøllestad *et al.* 1988), suggesting that exhaustion from RIE may be due to lack of substrates for ATP resynthesis. However, a similar study showed only moderate substrate changes at exhaustion, where the mean PCr level was still 50 % of control (Sahlin *et al.* 1992), arguing against the contention of an 'energy crisis' at exhaustion from RIE. The inconclusive role of energy metabolism in exhaustion from low-force RIE may partly be due to the poor time resolution obtainable in muscle biopsy studies. The second aim of this paper was therefore to re-examine the metabolic changes associated with exhaustion.

Recent methodological advancements allow a closer and parallel study of the temporal pattern of metabolic and mechanical factors in fatigue and exhaustion in human muscle *in vivo* (Edwards, Gibson, Roberts, Clague & Martin, 1992). In the present study chemical changes in the vastus lateralis muscle were closely monitored by high-resolution topical ^{31}P NMR. The force responses in the quadriceps muscle to electrical stimulation were monitored simultaneously during and after exercise using a standardized computer-programmed stimulation regime (programmed stimulation myogram, PSM) (Cooper, Edwards, Gibson & Stokes, 1988). E–C coupling failure during fatigue may be observed as a shift in the force–frequency relationship where force from low-frequency stimulation has been shown to decline more than from high-frequency stimulation (Edwards, Hill, Jones & Merton, 1977). This 'low-frequency' fatigue may be observed from the 20:50 Hz force ratio during the PSM (Cooper *et al.* 1988; Gibson, Cooper, Stokes & Edwards, 1988), allowing us to determine whether the E–C coupling was impaired during and after exercise in the present experiment.

Preliminary presentations of the present results have been given elsewhere (Gibson, Saugen, Martin & Vøllestad, 1993; Vøllestad, Saugen, Gibson, Martin & Edwards, 1993).

METHODS

Subjects

Eight males drawn from a population of healthy volunteers (age, 23–33 years; mean age, 27 years) participated as experimental subjects. None of the subjects was specifically trained for sport activities, but all were recreationally active. The subjects were familiar with the experimental procedures and had given their informed consent before participation. The procedures were approved by the ethics committee of the Royal Liverpool University Hospital, Liverpool.

Experimental design

The experiments were performed with the subject in a supine position inside a 48 cm bore whole body 1·5 T superconducting magnet. The exercise was carried out with one leg, which was positioned over a polystyrene wedge-shaped support, giving a 100 deg knee angle. In order to minimize body and leg movement the hips were strapped to the bed. Isometric knee extension force was measured by a custom-made strain gauge connected via an aluminium rod and an inflexible padded strap placed around the ankle. The subjects had a visual feedback of force from a light-emitting diode (LED) array placed behind the subject and viewed with a mirror.

Force was recorded on a UV oscillograph (SE 3006/DL, SE Laboratories, Feltham, UK) placed in the control room. The maximal force-generating capacity was determined at the beginning of each experiment, where each subject performed three consecutive MVCs with 2 min rest between. MVC force was determined as the highest force that could be held for 2 s. The exercise consisted of repetitive isometric knee extensions at 40 % MVC for 6 s, with 4 s rest between, carried out until exhaustion. Exhaustion was defined as not being able to maintain target force for the required 6 s. After exhaustion the subjects rested in the magnet for 30 min, during which test contractions were performed to monitor force-generating capacity.

Voluntary and electrically stimulated force-generating capacities were monitored in test sequences sequentially consisting of: 3 s MVC, 7 s rest, 6 s 40 % MVC, 4 s rest, a 6 s stimulated contraction pattern (PSM) and 4 s rest. Three test sequences were performed before exercise with 5 min rest between. During exercise, test sequences were performed at 1 and 5 min and thereafter every 5 min to exhaustion. During the 30 min post-exercise recovery period test sequences were performed at 2, 5, 10, 15, 20, 25 and 30 min. Due to displacements of the stimulation electrodes in one subject, stimulated force data were obtained in only seven subjects.

Muscle stimulation

The computer-controlled PSM consisted of a sequential train of impulses (square wave, 50 μs) delivered at 1, 10, 20, 50, 100 and 1 Hz for 1 s each (2 s for 10 Hz to obtain plateau force). The stimulation voltage was adjusted to a level that produced the largest twitch response without causing painful effects. The electrodes used for stimulation consisted of 10 cm × 10 cm defibrillator pads (3M defibrillator pads, St Paul, MN, USA) placed on the skin with conducting electrode gel as an interface. These were placed at a distal and proximal position over the anterolateral portion of the thigh. Stimulator leads passed from the control room via filters (low-pass 100 dB attenuation, 100 kHz to 10 GHz, 600 Ω line impedance), and were attached to the defibrillator pads using 2 cm × 2 cm copper foil pads. Polythene was placed over the electrode arrangement to prevent drying of the electrodes and the assembly was strapped firmly to the thigh with elastic bandages.

^{31}P acquisition and analysis of spectra

The experiments were carried out in a 1·5 T GE Signa MR spectrometer (General Electrics Medical Systems, Milwaukee, WI, USA) with a one-pulse ^{31}P NMR acquisition. An elliptical surface coil measuring 10 cm × 6 cm, constructed 'in-house' and tuned to a frequency of 25·86 MHz for phosphorus, was placed (during contraction) over the medial part of the belly of the vastus lateralis muscle. The coil was securely fastened to the skin with surgical tape. The coil and the leg were held in a fixed position in the magnet. The B_1 field homogeneity was optimized by off-resonance proton-shimming on the muscle water peak. ^{31}P spectra were obtained with a 9 s interpulse delay giving partially saturated conditions. The spectral width was ±2000 Hz and the number of data points was 2000.

The free-induction decays (FIDs) were processed with baseline correction, apodized with 6 Hz Lorenztian line broadening and Fourier-transformed into spectra. The areas under the phosphorus peaks were calculated by means of a Marquardt algorithm after a manual baseline correction. Due to the considerable variation in both PCr content between different muscle fibre types (Edström, Hultman, Sahlin & Sjöholm, 1982), and differences in fibre-type distribution between subjects (Lexell & Taylor, 1989), the high-energy phosphate data have been presented relative to the values obtained in unfatigued muscle.

Data analysis and statistics

To minimize noise due to movement artefacts and changes in signal strength all calculations of high-energy phosphate and P_i levels were based on the ratio between the area of the respective peaks and total phosphate peak area (PCr + P_i + γATP). The saturation factor was assumed to remain constant due to the constant pulse delay throughout the experiment, allowing all phosphate compound levels to be given relative to pre-exercise values. pH was calculated from the chemical shift, δ, (in p.p.m.) of the P_i peak relative to the PCr peak (Arnold, Matthews & Radda, 1984): $pH = 6{\cdot}75 + \log[(\delta - 3{\cdot}27)/(5{\cdot}69 - \delta)]$. In an attempt to monitor high-energy phosphate fluctuations during the duty cycle, the spectra were acquired every 9 s in a duty cycle of 10 s, so that a set of ten scans would provide information on duty cycle variation. The single-pulse spectra signal : noise ratio was too low to provide interpretable results on duty cycle variations in PCr and P_i. Chemical data have therefore been averaged over ten scans to minimize random fluctuations due to sampling time.

Statistical analysis was carried out using Sigmastat (Jandel Corp., Erkrath, Germany). Statistical procedures used for data inference are stated in the text where appropriate. One-way repeated measures ANOVA was used for comparison of means, and a significance level of 5 % was adopted for testing hypotheses. For the detection of differences between means within groups Student–Newman–Keuls *post hoc* test was adopted where appropriate. Data are given as mean values ± S.E.M.

RESULTS

Voluntary and stimulated force

Pre-exercise force levels during voluntary and electrically stimulated contractions are given in Table 1, which shows the relative changes in force at exhaustion (40 ± 6 min) and after recovery. The highest force during electrical stimulation was obtained at 100 Hz, and was 52 % of mean MVC force in unfatigued muscle. A gradual development of fatigue was seen with only small variations in time course between subjects. At exhaustion MVC had declined by 44 ± 5 % ($n = 7$). An initial rapid phase of recovery to 68 ± 7 % was seen during the first 5 min after exercise, followed by a slower recovery rate to 79 ± 4 % of control during the remainder of the 30 min post-exercise period. The force response during electrical stimulation declined to a larger degree than MVC, as shown in Fig. 1 and Table 1. The magnitude of force decline during electrical stimulation was inversely related to the stimulation frequency, with the largest force reduction seen from 10 Hz stimulation, where the exhaustion value was 10 ± 2 % of control. Accordingly the 20 : 50 Hz force ratio declined from 0·76 ± 0·02 in unfatigued muscle to 0·33 ± 0·01 at exhaustion (Fig. 1). During post-exercise recovery high-frequency force was more rapidly restored than low-frequency force. The 20 : 50 Hz ratio therefore continued to decline, initially to a minimum value of 0·24 ± 0·03 after 10 min recovery, followed by a gradual slow increase during the next 20 min to near exhaustion value.

Chemical changes during exercise and recovery

There was a significant decline in the total phosphate peak area (P_i + PCr + γATP) during exercise, reading 84 ± 8 % ($n = 8$) at exhaustion compared with pre-exercise values. After 30 min post-exercise recovery the total phosphate peak area returned to 92 ± 9 % of pre-

Table 1. *Force responses from voluntary and electrically stimulated activation before and after repetitive isometric exercise at 30 % MVC*

	Pre-exercise (N)	Exhaustion (%)	Recovery (%)
MVC	352 ± 31	56 ± 5*	79 ± 4*†
10 Hz	39 ± 5	10 ± 2*	18 ± 2*†
20 Hz	130 ± 13	18 ± 3*	33 ± 3*†
50 Hz	174 ± 14	39 ± 5*	66 ± 3*†
100 Hz	183 ± 14	37 ± 7*	71 ± 4*†
20:50 Hz ratio	0·76 ± 0·02	0·33 ± 0·01*	0·37 ± 0·02*

Values are means ± S.E.M., $n = 7$. Force before exercise is given as absolute force level. At exhaustion and recovery the force responses are given relative to pre-exercise value. The 20:50 Hz force ratio was calculated from absolute force values obtained by electrical stimulation at 20 and 50 Hz. *Significantly different from pre-exercise; †significantly different from exhaustion; $P < 0·05$; one-way ANOVA, repeated measures design.

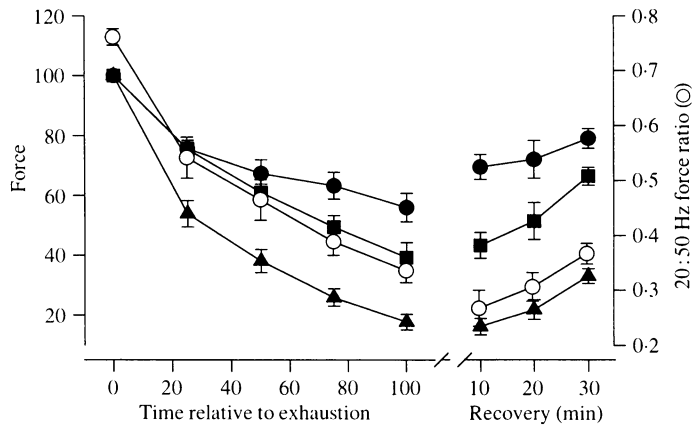

Fig. 1. Change in force-generating capacity during maximal voluntary contractions (●) and stimulated contractions at 20 Hz (▲) and 50 Hz (■) stimulation. The change in ratio between 20 and 50 Hz force is also plotted (○). Exercise data are plotted on a scale relative to exhaustion, while the recovery data are plotted in real time. Force data are given relative to pre-exercise control values, while the 20:50 Hz force ratio is given as the ratio between absolute force values. Data are given as means ± S.E.M. for 8 and 7 subjects for MVC and stimulated contraction, respectively.

exercise values, which was still significantly different from control. However, the ratio between the P_i + PCr peak area and the total phosphate peak area (P_i + PCr + γATP) was unchanged throughout the exercise ($P = 0·49$), and the relative area of the γATP peak was not different at the beginning and end of the experiment ($P = 0·48$). The fall in total phosphate peak area was therefore probably caused by exercise-induced fluid accumulation in the vastus lateralis muscle (Sjøgaard, Adams & Saltin, 1985), in keeping with a 15 % decline in protein concentration seen during 30 % MVC RIE (Vøllestad *et al.* 1988). Hence, our chemical data, determined relative to total phosphate peak area, probably reflect the actual changes in relative substrate and metabolite levels.

A decline in PCr, with a concomitant increase in P_i was seen from the onset of exercise in all subjects. However, in contrast to the homogeneous change in force responses, the magnitude

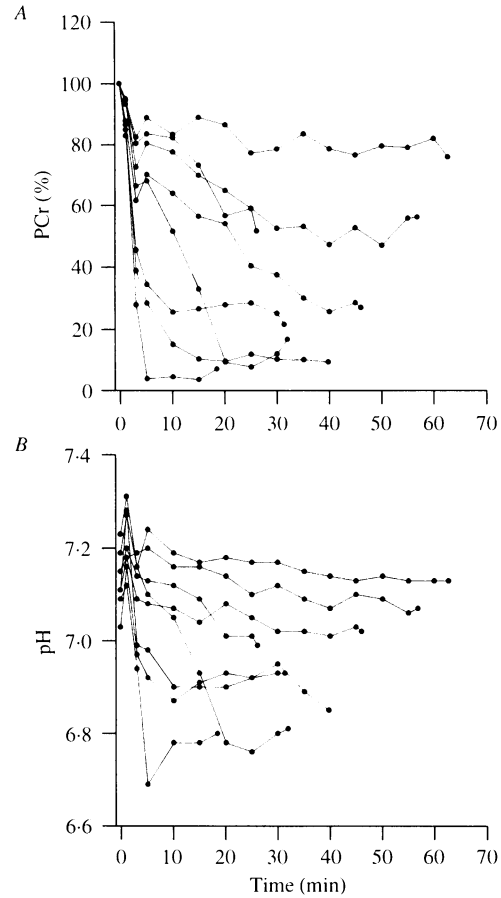

Fig. 2. Individual changes in PCr (*A*) and pH (*B*) during submaximal repetitive intermittent exercise until exhaustion. PCr content is given relative to pre-exercise control values. Each point represents the mean of 10 scans (taken over 1·5 min), giving a duty cycle mean value. Lines have been drawn between points relating to an individual to improve the clarity of presentation. pH is calculated from the horizontal shift in P_i peak relative to the PCr peak (see Methods for details on calculation).

and temporal pattern of chemical changes varied widely between subjects, as shown in Fig. 2. In four subjects PCr declined rapidly during the first 5–10 min before levelling off at a low level. In these subjects exercise could be continued for a further 15–20 min with PCr levels close to depletion. In other subjects a steady and more moderate change in PCr throughout the exercise period was seen. The mean PCr level at exhaustion was 35 % of pre-exercise values, with a range of 7–76 %. In the individual subjects the magnitude and time course of the P_i change showed a mirror image of the PCr level during exercise and recovery. The individual variations in metabolic response pattern were also clearly seen after the pre-exercise test sequences (see Methods), which induced a decline in PCr level varying between 5 and 63 % of control. The magnitude of this change in PCr was linearly correlated (regression coefficient, $r^2 = 0{\cdot}65$) with the corresponding individual PCr level seen after 3 min of RIE (40 ± 7 % of control).

Table 2. *Chemical changes during repetitive isometric exercise at 40 % MVC*

	PCr (%)	ATP (%)	pH
Rest	100	100	7·13 ± 0·02
Exercise time			
25 %	51 ± 10	101 ± 5	7·00 ± 0·06
50 %	40 ± 10	109 ± 7	6·99 ± 0·05
75 %	34 ± 9	107 ± 8	6·95 ± 0·05
Exhaustion	35 ± 9	102 ± 8	6·95 ± 0·04

Exercise time is given as a percentage of endurance time. PCr and ATP levels are presented as percentages of their respective pre-exercise values. All high-energy phosphate levels were first calculated relative to the total phosphate peak area (P_i + PCr + γATP; see Methods).

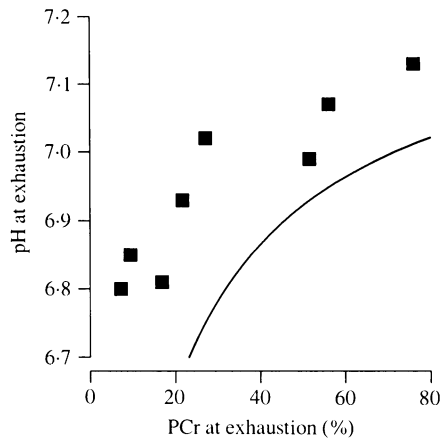

Fig. 3. Individual values of pH (■) at exhaustion as a function of the corresponding PCr value (as a percentage of pre-exercise value). pH and PCr data represent means of the last 10 scans before exhaustion. The curved line is drawn according to the equations of a model developed by Sahlin and co-workers (Sahlin *et al.* 1975; Harris *et al.* 1977), and shows the predicted relationship between pH and PCr after sustained isometric contractions held to exhaustion.

The initial hydrolysis of PCr led to a minute increase in pH of 0·16 pH units to a peak value of 7·29 ± 0·04 after 1 min of exercise. Figure 2 shows how the individual changes in pH thereafter reflected the time course of the changes in PCr. Hence, pH declined in all subjects, with the most pronounced decline in the individuals with the largest fall in PCr. In those subjects where PCr levelled off at a low plateau, pH also remained unchanged as exercise was continued. At exhaustion we observed a linear relationship between PCr and pH as shown in Fig. 3 ($r^2 = 0{\cdot}83$, $P = 0{\cdot}002$). The continous line in Fig. 3 shows the relationship between pH and PCr after exhaustive sustained isometric contractions as predicted by a model from Sahlin *et al.* (1975) and Harris *et al.* (1977). Even though RIE caused a substantial depletion of PCr in some subjects, pH did not decline to the same extent as during more intense contractions.

Table 2 summarizes the exercise-induced changes in PCr, ATP and pH levels. The data represent the means of ten scans (taken over 1·5 min), and exercise time is given relative to exhaustion, to enable comparison despite the differences in endurance. Although exercise was continued with nearly depleted PCr in some subjects, ATP did not change significantly during

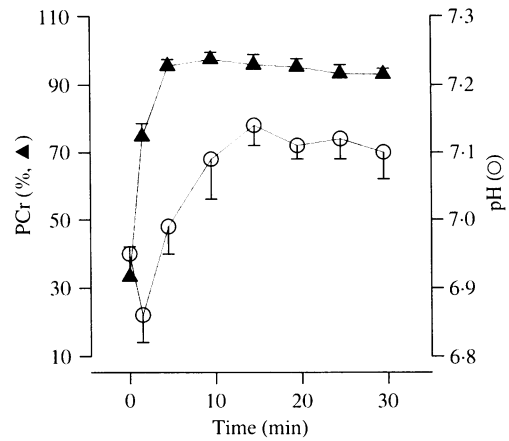

Fig. 4. Changes in PCr level and pH during recovery after exhaustion from repetitive isometric exercise. PCr content is given relative to pre-exercise control value. Data are averaged over 10 scans (1·5 min) prior to the times indicated on the plot and are given as means ± S.E.M. for 8 subjects.

the exercise, being 102 ± 8 % of control at exhaustion. During the last quarter of the exercise no change was seen in the mean values of PCr, ATP or P_i content, or in pH (cf. Table 2). Exhaustion was thus not accompanied by any sudden final changes in high-energy phosphate or metabolite chemistry.

After exhaustion PCr recovered to 95 ± 2 % of pre-exercise values within the first 5 min and then remained stable for the rest of the 30 min recovery period, as shown in Fig. 4. The initial rapid PCr resynthesis during recovery was associated with a continued decline in pH to a nadir value of 6·79 ± 0·07 after 1·5 min recovery. Thereafter pH recovered more slowly than PCr, reaching 7·09 ± 0·06 after 10 min, a value not significantly different from control. Comparing Figs 1 and 4, the dissociation of the time courses for force and chemical recovery is clearly seen. While the high-energy phosphate levels and pH had recovered to near control values within the first 10 min after exercise, the force responses were still less than fully recovered after 30 min. The dissociation between high-energy phosphate and metabolite chemistry, and changes in mechanical response were further emphasized by a complete lack of correlation between individual chemical and force data at any time during the present experiment.

DISCUSSION

The present study showed a clear dissociation between a similar and near-linear change in force-generating capacity between subjects, and a non-linear and highly variable pattern of changes in metabolite levels. In some subjects RIE could be continued for 10–15 min with PCr levels nearly depleted, without further changes in pH or ATP. The decline in MVC was accompanied by the development of low-frequency fatigue, possibly indicating an impairment of E–C coupling.

Metabolic and mechanical responses during repetitive isometric exercise

MVC and 50 Hz force declined almost linearly from the onset of the 40 % MVC RIE in all subjects. The homogeneous force responses and temporal pattern of force decline are similar to those seen in earlier studies of submaximal voluntary (30 % MVC) knee extensor RIE

(Vøllestad *et al.* 1988, 1990; Sahlin *et al.* 1992; Sejersted, Saugen & Vøllestad, 1993; Mengshoel, Saugen, Førre & Vøllestad, 1995). Pre-trials at 30% MVC demonstrated considerably longer endurance times in the supine position with a 100 deg knee angle than when the exercise was carried out sitting in a chair (85–90 deg knee angle). Whether this was due to a change in body position, or to changes in muscle length is not known, but fatigue has been shown to develop more rapidly in shortened muscle than at optimal muscle lengths in the human ankle dorsiflexor muscles (Fitch & McComas, 1985). Target force was therefore increased to 40% MVC in the present study to obtain endurance times comparable to those seen in the 30% MVC studies.

Contrary to the force responses, the metabolic response patterns, seen as changes in PCr, P_i and pH levels, varied widely between subjects. This result contrasts markedly with the observations of Vøllestad *et al.* (1988), where all subjects showed only minor changes in metabolite levels until the last few minutes before exhaustion from 30% MVC RIE (duty cycle: 6 s contraction, 4 s rest). Only one subject developed fatigue without substantial changes in PCr content and pH in the present experiment. The other seven subjects showed a rapid but variable initial decline in PCr content and pH, followed by a levelling off towards exhaustion. This latter response pattern is more in keeping with the results of Sahlin *et al.* (1992) from a study of 30% MVC RIE with a duty cycle of 10 s contractions with 10 s rest between. The variation in metabolic response pattern was most clearly seen during the first 3 min of RIE, where the decline in PCr level varied between 18 and 72% of control. One explanation may be that the target force (% MVC) varied between subjects, which would be the case if MVC was incorrectly determined. However, the procedure for determining MVC was similar to earlier experiments, where maximal activation was readily obtained in the quadriceps muscle during brief maximal voluntary isometric contractions (Bigland-Ritchie *et al.* 1986*b*; Vøllestad *et al.* 1988). Other investigators have shown that in healthy subjects the MVC force in the quadriceps muscle is 95–100% of the maximal evocable force (Gandevia, Allen & McKenzie, 1995). Thus it is unlikely that the differences in voluntary activation were sufficient to cause the variable metabolic responses.

It is of note that the metabolic responses to the pre-exercise test sequences also varied considerably between subjects, and a closer look at the individual values shows that the last pre-exercise test sequence led to a decline in PCr level that was comparably large, and linearly correlated with that seen after 3 min of RIE. During the test sequence the muscle activation pattern, involving both maximal voluntary motor drive and electrical stimulation, was markedly different from that seen during submaximal RIE (Bigland-Ritchie, Cafarelli & Vøllestad, 1986*a*). The high correlation between the initial PCr decline during RIE and the pre-exercise test sequences therefore argues in favour of individual metabolic properties being the cause of the variation in metabolic response seen during RIE. The human vastus lateralis muscle displays considerable variation in fibre-type distribution between subjects (Lexell & Taylor, 1989), and the metabolic response patterns during activity vary significantly between fibre types (Meyer, Brown & Kushmerick, 1985). Hence, the variable metabolic response patterns could possibly be related to fibre type. This line of reasoning is in keeping with the large inter-subject differences in the rate of energy turnover observed during 30% MVC RIE (Vøllestad *et al.* 1990; Saugen & Vøllestad, 1996).

Fatigue and recovery from repetitive isometric exercise

Fatigue, seen as a decline in MVC, may be caused by changes in one or several steps in a complex chain of central and peripheral events (reviewed in Westerblad, Lee, Lännergren &

Allen, 1991; Fitts, 1994). Earlier investigations of fatigue from submaximal voluntary RIE demonstrate a capability to maximally activate the quadriceps muscle by voluntary effort throughout the exercise period and during recovery, arguing against motivational or central factors causing the fatigue (Bigland-Ritchie *et al.* 1986*b*; Vøllestad *et al.* 1988; Mengshoel *et al.* 1995). The question of whether fatigue in the present experiment was caused by a progressive failure of neuromuscular transmission could not be examined. However, in a similar experiment on the adductor pollicis muscle no evidence was found for neuromuscular transmission failure despite a reduction of up to 50 % in MVC (Bigland-Ritchie *et al.* 1986*b*). In this latter study the maintained M waves also showed that the action potential propagation remained intact during this kind of exercise. These findings are in keeping with later investigations showing that motor units fire at low rates (12–15 Hz) during 30 % MVC RIE (Bigland-Ritchie *et al.* 1986*a*). Hence, it seems likely that the cause of fatigue during submaximal RIE resides within the muscle itself.

In the present experiment the force-generating capacity was measured both as MVC force, and as the force response to a computer-programmed electrical stimulation regime (PSM), frequently used in fatigue studies on human muscle (Cooper *et al.* 1988; Gibson *et al.* 1988). The force data demonstrate two aspects of mechanical changes during muscle contraction, namely fatigue, seen as a decline in force during maximal voluntary and electrically stimulated activation, and frequency dependence with a relatively larger decline in force from low-frequency compared with high-frequency electrical stimulation, causing a shift in the force–frequency relationship (low-frequency fatigue, Edwards *et al.* 1977).

Fatigue from isometric contraction in human muscle has mostly been studied during ischaemic or high-force contractions, where the decline in maximal force is associated with an accumulation of P_i and H^+ ions (Ahlborg *et al.* 1972; Sahlin *et al.* 1975; Harris *et al.* 1977; Kushmerick & Meyer, 1985). The present results show that a similar P_i increase may occur during RIE, while H^+ levels rise only marginally. The minor pH changes indicate that the rapid reperfusion of the muscle during the intermittent rest periods (Vøllestad *et al.* 1990) enables sufficient aerobic ATP resynthesis, in keeping with previous results from 30 % MVC RIE, showing a very moderate rise in muscle lactate (Vøllestad *et al.* 1988), as well as little release of lactate from the muscle (Vøllestad *et al.* 1990; Sahlin *et al.* 1992).

In most subjects P_i increased to levels that have been shown to have a marked inhibitory effect on maximal force in skinned rabbit and frog muscle fibre preparations (Donaldson & Hermansen, 1978; Kentish, 1986). However, the influence of P_i on maximal force during submaximal RIE may be questioned for several reasons. First, a comparison of the temporal patterns of metabolic and mechanical factors during the exercise shows a clear dissociation. Force declined only marginally during the initial rapid chemical changes, and continued to decline after PCr content and pH had levelled off (cf. Figs 1 and 2). Second, a dissociation between chemistry and force was also seen as a lack of correlation between the chemical and mechanical changes at exhaustion. Finally, during the post-exercise recovery period P_i declined to near control values during the first 5 min, while MVC remained below control values throughout 30 min post-exercise recovery. This latter result is in contrast to the rapid and parallel recovery of maximal force and P_i seen after sustained contraction in human muscle (Cooper *et al.* 1988; Baker, Kostov, Miller & Weiner, 1993), but in keeping with earlier results on recovery from submaximal RIE (Sahlin *et al.* 1992; Baker *et al.* 1993). Hence, our data support the hypothesis that fatigue from submaximal voluntary RIE is mainly due to causes other than those of P_i and H^+ accumulation on maximal Ca^{2+}-activated tension (Vøllestad *et al.* 1988).

The present study showed a faster decline in force generated by 20 Hz stimulation compared with 50 Hz stimulation, and this difference became markedly larger in the post-exercise recovery period (cf. Fig. 1 and Table 1). A similar phenomenon has been reported during and after RIE at 30 and 45 % MVC and after intense exercise (Edwards *et al.* 1977; Sejersted *et al.* 1993). Edwards *et al.* (1977) suggested that this disproportionate decline in force from low-frequency stimulation was caused by a reduced release of Ca^{2+} from the sarcoplasmic reticulum per action potential. This hypothesis was later confirmed by Allen, Westerblad and others (reviewed by Westerblad *et al.* 1991), who demonstrated that fatigue induced by repetitive contractions results in reduced Ca^{2+} release. This occurs in spite of normal action potentials and a homogeneous accumulation of Ca^{2+} in the muscle cells, indicating that activation of the muscle fibres is adequate. The available evidence thus suggests that the reduced Ca^{2+} release is caused by impaired E–C coupling, i.e. the transfer of signal from the depolarization of t-tubules to release of Ca^{2+} (Chin & Allen, 1996). Because saturation of Ca^{2+} at the troponin site is only achieved during fully fused tetanic contractions (i.e. at high excitation rates), a reduction in Ca^{2+} release will primarily affect force elicited at lower stimulation frequencies. Hence, the decline in the 20:50 Hz ratio seen in the present study may indicate that fatigue from submaximal RIE is associated with impaired E–C coupling, as first suggested by Vøllestad *et al.* (1988).

The gradual increase in central motor drive during RIE is due both to increased motor unit firing rate and to sequential recruitment of unfatigued motor units (Bigland-Ritchie *et al.* 1986*a*). Still, firing rates were in the low range (10–15 Hz), and development of low-frequency fatigue is therefore likely to have a pronounced effect on motor unit force output (Edwards *et al.* 1977). The large increase in central motor drive seen during low-force RIE may therefore be due, at least partly, to E–C coupling impairment.

In a previous study it was shown that about half the motor unit population was recruited from the onset of submaximal voluntary RIE, and that the motor unit firing rate is relatively low (10–15 Hz) (Bigland-Ritchie *et al.* 1986*a*). As fatigue developed, target force was maintained by a gradual recruitment of unfatigued motor units, and at exhaustion all but a few units were needed to generate target force. The fall in tetanic or MVC force may underestimate the actual reduction in force generated by the active motor units during submaximal target force contractions, since these are subject to low-frequency fatigue. The need for a large increase in muscle activation may therefore possibly be explained by a reduction in E–C coupling efficiency.

Exhaustion

Exhaustion was defined as the point where target force could no longer be maintained for the required 6 s (Bigland-Ritchie *et al.* 1986*b*; Vøllestad *et al.* 1988). During 30 % MVC RIE, Vøllestad *et al.* (1988) reported that exhaustion was associated with sudden metabolic changes, seen in all subjects as a rapid decline in substrates and accumulation of P_i. It was suggested that exhaustion from submaximal voluntary RIE may be associated with an 'energy crisis' possibly involving a lack of substrates for immediate ATP resynthesis. The present data show a strikingly different picture, where neither force-generating capacity nor metabolite levels changed suddenly as exhaustion was approached (cf. Figs 1 and 2 and Table 2). Instead the rapid decline in PCr seen in most subjects occurred during the first minutes of the exercise. Even in subjects where PCr was nearly depleted, exercise could be continued for 15–20 min without a decline in ATP, strongly suggesting that exhaustion was unrelated to high-energy phosphate metabolism (cf. Figs 2 and 3).

Vøllestad *et al.* (1988) also observed an increased rate of muscle glycogen breakdown and a marked rise in muscle lactate towards exhaustion, indicating that glycolytic activity and anaerobic energy release were increased to meet the ATP demand. The present observation of a constancy of pH during the last minutes of exercise does not support this view. Instead pH remained high and at exhaustion was considerably higher than that seen for similar high-energy phosphate changes after sustained knee extensor contractions and intense cycling (Sahlin *et al.* 1975; Harris *et al.* 1977). More recent investigations show that although the ATP demand increased twofold during 30 % MVC RIE (Vøllestad *et al.* 1990; Sahlin *et al.* 1992; Saugen & Vøllestad, 1996), the calculated metabolic rate was still well below the aerobic capacity of the quadriceps muscle (Saltin, Henriksson, Nygaard & Andersen, 1977). This line of reasoning further refutes the possibility of an 'energy crisis' at exhaustion.

We can only speculate on which other factors may have been involved in limiting endurance. During voluntary exercise the ability to overcome the perception of increasing effort is of importance in reducing the influence of central and motivational factors. In a similar study (Mengshoel *et al.* 1995) the exertion score on a Borg category scale increased gradually throughout the exercise period, approaching maximal scale value at exhaustion. However, target force was still well below the MVC. This is in keeping with the comments given by the subjects in the present study. Clearly, further studies are needed to understand whether exhaustion from submaximal voluntary RIE is caused by peripheral, central or motivational factors.

Conclusion

The clear temporal dissociation between metabolic response and changes in force-generating capacity support the hypothesis that fatigue from submaximal voluntary RIE is unrelated to metabolite accumulation. Exercise could, in spite of nearly depleted PCr levels, be continued without further pH changes, and exhaustion was not associated with any sudden final changes in high-energy phosphates or pH. Hence, exhaustion was apparently not caused by lack of substrates for ATP resynthesis. The frequency-dependent decline in force-generating capacity indicates that fatigue was associated with E–C coupling impairment. Development of low-frequency fatigue may contribute to the gradual increase in central motor drive needed to maintain target force during low-force RIE.

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