

³¹P Nuclear Magnetic Resonance Studies of High Energy Phosphates and pH in Human Muscle Fatigue

Comparison of Aerobic and Anaerobic Exercise

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Abstract

The goal of these experiments was to investigate the relationship of ATP, phosphocreatine (PCr), inorganic phosphate (Pi), monobasic phosphate (H_2PO_4^-), and pH to human muscle fatigue. Phosphates and pH were measured in adductor pollicis using ³¹P nuclear magnetic resonance at 2.0 Tesla. The force of muscle contraction was simultaneously measured with a force transducer. The effects of aerobic and anaerobic exercise were compared using two exercise protocols: 4 min sustained maximal voluntary contraction (MVC) and 40 min of repeated intermittent contractions (75% MVC). The sustained maximal contraction produced a rapid decline of MVC and PCr, and was accompanied by a rapid rise of Pi, H⁺, and H_2PO_4^- . Intermittent exercise produced steady state changes of MVC, pH, and phosphates. No significant changes of ATP were found in either protocol. During fatiguing exercise, PCr and Pi had a nonlinear relationship with MVC. H⁺ showed a more linear correlation, while H_2PO_4^- showed the best correlation with MVC. Furthermore, the correlations between MVC and H_2PO_4^- were similar in sustained ($r = 0.70$) and intermittent ($r = 0.73$) exercise. The highly significant linear relationship between increases of H⁺ and H_2PO_4^- and the decline of MVC strongly suggests that both H⁺ and H_2PO_4^- are important determinants of human muscle fatigue.

Introduction

The mechanism by which exercise produces muscular fatigue is unclear. Several factors have been proposed including alteration of muscle membrane function (1, 2), reduction of high energy phosphates (3–5), accumulation of H⁺ (6–10), and impairment of excitation–contraction coupling (11–13). Evidence for each of these has been found using in vitro animal preparations, but the relative importance of these mechanisms in human fatigue (defined as a decline of maximum force-generating capacity) remains uncertain. Previously, the role of high energy phosphates and pH was investigated using rapidly frozen muscle biopsies (10, 14–16). Recently, it was suggested from in vitro animal experiments that H_2PO_4^- might also be a causative factor in fatigue (17–19). To avoid using invasive muscle biopsy, several investigators have used ³¹P nuclear magnetic resonance¹ (NMR) to monitor noninvasively phos-

phocreatine (PCr), inorganic phosphate (Pi), ATP, and pH in animal (4, 20–22) and human muscle (13, 23–27). These studies have shown that in human muscle, exercise causes depletion of PCr with concomitant accumulation of Pi and H⁺ (24–27). ATP concentrations are maintained at a constant level unless the exercise is severe and PCr is depleted (27).

Studies in this laboratory were designed to characterize the relationship between various metabolic parameters and fatigue in human muscle. Techniques of simultaneously measuring force production, electromyography, and metabolite levels by ³¹P NMR were developed (13). The initial experiments focused on the effects of a voluntary, sustained, maximal 4-min isometric contraction of the human adductor pollicis, a muscle that has been extensively studied by others (11, 28). The results confirmed that fatiguing exercise causes PCr depletion and increases of Pi and H⁺. During exercise, the decline of force (fatigue) roughly correlated with all three parameters.

One problem with the interpretation of the previous studies is that a sustained maximal contraction produces a rapidly changing state of muscle function and metabolism. To obtain adequate spectra, ³¹P NMR signals must be accumulated over a period of at least 1 min; thus, the data represent a time-averaged mean during each 1-min acquisition period. In addition, a prolonged maximal contraction is a less common exercise in everyday life than intermittent contraction. Furthermore, maximal sustained contraction markedly reduces blood flow—producing ischemia, which stimulates anaerobic metabolism. Intermittent exercise allows intermittent blood flow, permitting aerobic metabolism. The purpose of the present experiments was to characterize and compare the metabolic and physiological effects of sustained anaerobic exercise with the effects of intermittent aerobic exercise. Intermittent exercise studies were designed to produce different steady state levels of fatigue (defined as a fall of maximum voluntary contraction [MVC]), permitting steady state NMR measurements. The major finding of these experiments is that the decline of maximum force best correlates with both H⁺ and H_2PO_4^- , suggesting that alterations of these compounds are causative factors in human muscle fatigue.

Methods

All studies were performed on the adductor pollicis of normal human volunteers who provided informed consent approved by the Children's Hospital of San Francisco and The University of California, San Francisco, Committees on Human Experimentation. The experimental

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1. Abbreviations used in this paper: MVC, maximal voluntary contraction; PCr, phosphocreatine; Pi, inorganic phosphate.

methods were similar to a previous report (13) except that a different NMR spectrometer was used. Therefore, the methodology will only be briefly summarized.

NMR and force measurements

The adductor pollicis rested on a teardrop-shaped single-turn surface coil. The spectrometer used for this study was a General Electric CSI-1 with a 2.0 Tesla 30-cm horizontal bore magnet. ^{31}P spectra were collected at 34.6 MHz in 1-, 2-, or 5-min blocks at a pulse rate of 80 repetitions per minute. The force transducer signal, measuring the force of an isometric voluntary contraction, was amplified (TECA electromyograph, TE-4 with AD6M) and fed into a calibrated analogue voltmeter to provide visual feedback for subjects. Before each exercise protocol was initiated, each subject performed a brief MVC. This control MVC indicated the maximum force of the unfatigued muscle (100% control MVC).

Exercise protocols

4-min sustained MVC. After collecting a 5-min control spectrum, 1-min spectra were acquired while the subject maximally activated the muscle against the force transducer. Superimposed single supramaximal motor nerve stimulation was accomplished every 30 s to verify that the muscle was maximally stimulated (twitch occlusion technique [28]). After the exercise, four 1-min, then four 2-min ^{31}P NMR spectra were collected, along with a 3-s MVC at the midpoint of each spectrum.

Intermittent contraction. Steady state levels of fatigue were induced by having a subject alternately contract and relax once within a 10-s period, and by repeating this exercise for 5 min at 75% of control MVC and at a constant duty cycle of contraction. Eleven such 5-min blocks of exercise were performed in succession without pause, each at a different duty cycle. After control measurements of MVC and ^{31}P NMR spectra, four different 5-min blocks were used to produce fatigue: (i) 6-s contraction/4-s rest; (ii) 7-s contraction/3-s rest; (iii) 8-s contraction/2-s rest; and (iv) 9-s contraction/1-s rest. At this time, lighter exercise was used to attain various steady states of recovery: (a) 5-s contraction/5-s rest; and (b) 3-s contraction/7-s rest, followed by three 5-min blocks of complete rest. Each 5-min block of exercise corresponded to one 5-min NMR spectrum. Analysis of 1-min spectral blocks indicated that a steady state was almost always reached after 1 min of exercise at any given duty cycle. For this reason, the data obtained during each 5-min period was averaged in the form of a single 5-min spectrum.

Analysis of data

Force measurements during the sustained exercise were averaged over the minute corresponding to each spectrum. During intermittent exercise, five (1/min) brief (3-s duration) MVC corresponding to each spectrum were averaged. A single MVC (3-s duration) corresponded to each spectrum during the recovery periods of both exercise protocols.

The baseline of the NMR spectra were flattened by convolution difference to subtract out the broad resonance due to the phosphorous components in bone and phospholipids. Peak areas were then integrated using GEMCAP software and a Nicolet 1280 computer (GE NMR Instruments Medical Systems Group, Fremont, CA).

During control experiments, slow-pulsed spectra (repetition time, 15 s) were obtained to determine saturation factors of the fast-pulsed spectra. Concentrations of metabolites were determined by assuming that control ATP equals 8.2 mM (27). Intracellular pH (pHi) values were determined from the chemical shift of Pi referenced to PCr (29). H_2PO_4^- concentrations were calculated from the Pi concentration and a pK of phosphoric acid equalling 6.75.

Data is expressed as the mean \pm standard error of the mean. Where indicated, data was analyzed using linear regression analysis.

Results

The effects of exercise on MVC, PCr, Pi, H^+ , and H_2PO_4^- (Fig. 1). Fig. 1, A and B, show typical ^{31}P NMR spectra obtained

during the anaerobic sustained exercise (1-min spectra) and the aerobic intermittent exercise (5-min spectra), respectively.

Fig. 1, C and D, compare the effects of sustained exercise (C) with intermittent exercise (D) on MVC, PCr, and Pi. 4-min sustained exercise (Fig. 1 C) produced a rapid decline of force to $32\pm 9\%$ of control after 3 min. PCr fell even more rapidly, reaching $8\pm 1\%$ of control after 2 min. The rise of Pi mirrored the fall of PCr.

Even though the intermittent exercise protocol was qualitatively and quantitatively different from that of the sustained exercise protocol, Fig. 1, C and D, show many similarities between the two types of exercise. The increasingly demanding intermittent exercise produced a decline of MVC to $27\pm 3\%$ of control at 20 min. PCr concentrations fell more rapidly, reaching $25\pm 5\%$ of control at 5 min and $15\pm 4\%$ of control at 10 min. The rise of Pi inversely paralleled the fall in PCr. Fig. 1 D clearly shows that the decline in MVC occurred more slowly than the decline of PCr. During the first 5 min of exercise, PCr rapidly fell to $25\pm 5\%$ of control, while force was maintained at $73\pm 2\%$ of control. From 10 to 20 min, PCr and Pi remained relatively constant, whereas MVC continued to drop.

Radda and colleagues (30) have reported muscle ^{31}P NMR data as $\text{PCr}/(\text{PCr} + \text{Pi})$. This analysis is based on the assumption that the total amount of PCr + Pi remains constant during exercise. For the sake of comparison, $\text{PCr}/(\text{PCr} + \text{Pi})$ was plotted as a function of MVC during sustained and intermittent exercise (data not shown). The relationship $\text{PCr}/(\text{PCr} + \text{Pi})$ is similar to that for PCr (Fig. 1). Chance and co-workers (24) have reported ^{31}P NMR data as the Pi/PCr (or sometimes PCr/Pi) ratio, which is considered to be an index of the "energy state." The data obtained for Pi/PCr (not shown) is similar to that for Pi, except that Pi/PCr rises to a greater extent and returns to control more quickly than Pi in both the sustained and intermittent protocols. In both sustained and intermittent exercise Pi/PCr initially rose, but then fell as MVC continued to decrease. Although ATP is used for muscle contraction, there was no significant change of ATP during both sustained or intermittent contraction (data not shown).

Fig. 2 shows changes of pHi (Fig. 2, A and B) and H_2PO_4^- (Fig. 2, C and D). Control pHi was 7.08 ± 0.04 . Fig. 2 A demonstrates a close relationship between changes of MVC and pHi during exercise. In contrast to PCr and Pi, both sustained and intermittent contraction produced a gradual fall of pHi which closely paralleled the drop in force. During sustained exercise, pHi dropped to 6.58 ± 0.09 after 2 min and remained at this value. After completion of the 4-min exercise pH did not begin to rise until 7 min, while PCr and Pi recovered much more quickly. During intermittent exercise, pHi gradually dropped to 6.55 ± 0.03 and then gradually returned to control values by 40 min; PCr and Pi had not returned to control values by this time. Fig. 2, C and D, also show that the rise of H_2PO_4^- mirrored the fall of MVC during exercise.

Radda and co-workers (30) previously reported the relationship between PCr or $\text{PCr}/(\text{PCr} + \text{Pi})$ and pH. In the present experiments, the relationship between pH and $\text{PCr}/(\text{PCr} + \text{Pi})$ was about the same during sustained and intermittent exercise (data not shown).

Relationship between MVC, ATP, PCr, Pi, H^+ , and H_2PO_4^- produced by sustained and intermittent exercise (Figs. 3–5). To ascertain the relationship of various metabolites (PCr, Pi, H^+ , and H_2PO_4^-) to muscle fatigue (defined as the fall of MVC), MVC was plotted as a function of each parameter. Linear

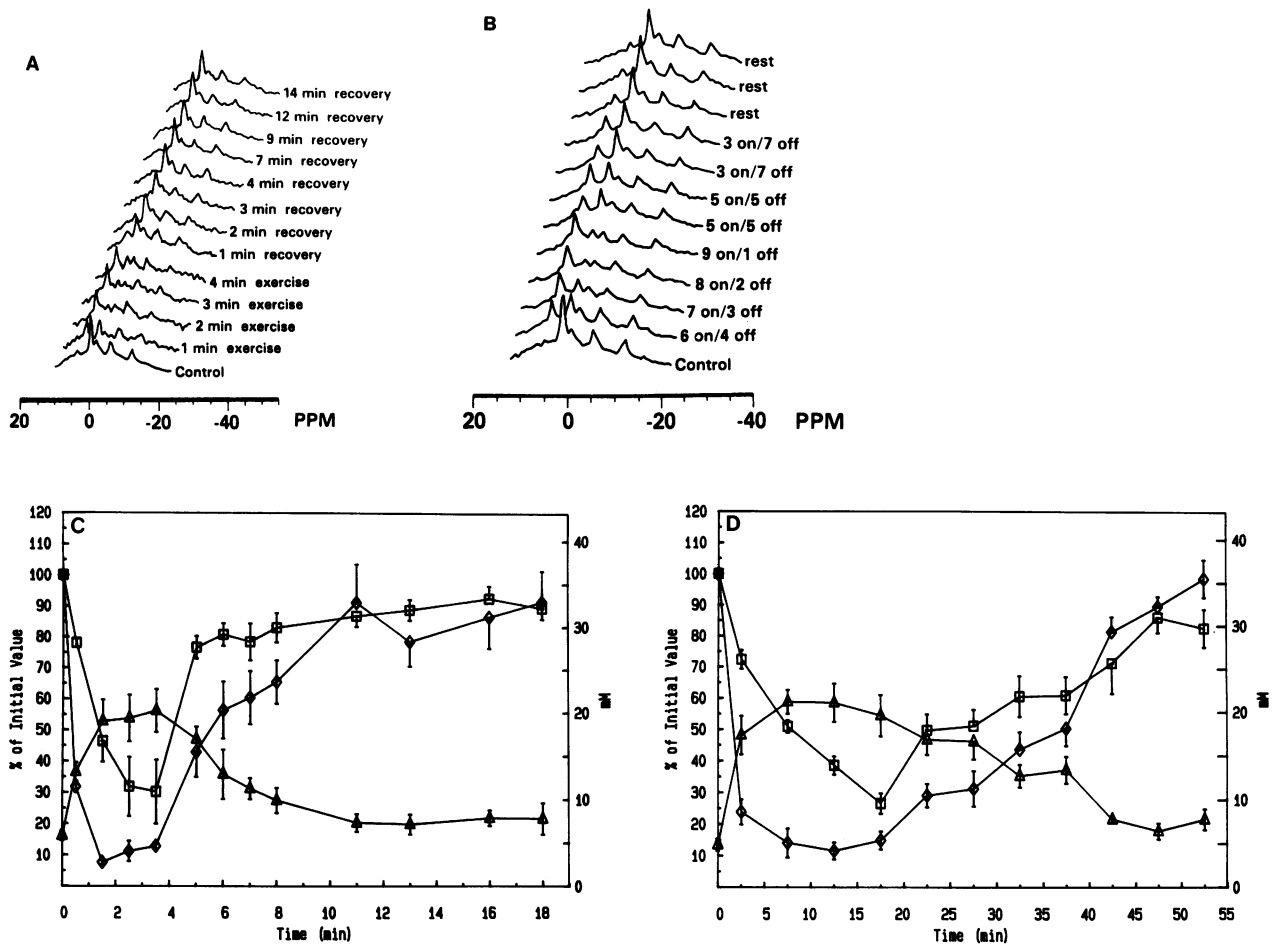


Figure 1. Stacked plot of ^{31}P NMR spectra obtained during 4 min sustained exercise (A) or intermittent exercise (B). Exercise protocols and NMR acquisition parameters are provided in Methods. MVC (\square), PCr (\diamond), and Pi (\triangle), plotted as a function of time during 4 min sustained (C) and intermittent (D) exercise.

regression analysis was performed when a roughly linear relationship was noted. In all cases, it was assumed that the metabolite was the independent variable and that MVC was the dependent variable.

Muscle exercise did not significantly change ATP; therefore, there was no relationship between changes of ATP and changes of MVC. Fig. 3 depicts the relationship between MVC and PCr during both sustained (A) and intermittent exercise (B). During the early period of sustained exercise, PCr dropped considerably, with relatively little change of MVC. Only when PCr fell below 10 mM did substantial fatigue occur. During intermittent exercise, a similar relationship was noted. PCr fell to ~ 10 mM with only a 20% decrease of MVC. As PCr became progressively depleted, there was a rapid drop of MVC. Therefore, the relationship between MVC and PCr was nonlinear. Because there was no change of ATP, there was no relationship between MVC and ATP in any form of exercise (data not shown).

There was also a nonlinear relationship between MVC and PCr/(PCr + Pi), which was very similar to that shown in Fig. 3. The relationship between MVC and Pi/PCr was also nonlinear.

In contrast to Fig. 3, there was a roughly linear inverse correlation between MVC and H^+ in Fig. 4. The correlation

coefficients (r) were 0.64 and 0.77 for sustained and intermittent exercise, respectively. Fig. 5 shows that there was also a roughly linear relationship between H_2PO_4^- and MVC ($r = 0.70$ and 0.71 for sustained and intermittent exercise, respectively). In Fig. 5A there is a very close, highly linear relationship between the mean values of MVC and H_2PO_4^- . This linear relationship was similar to that found between MVC and H^+ , but contrasted with the nonlinear relationships between MVC and PCr or Pi. These results suggest that H_2PO_4^- or H^+ are important determinants of human muscle fatigue.

Discussion

The first aim of the present experiments was to produce various steady state levels of fatigue (using intermittent exercise) in which maximum force, high energy phosphates, and pH were maintained for several minutes. This allowed correlations between MVC and steady state measurements of metabolites. It was anticipated that aerobic exercise (intermittent contraction) would be associated with less acidification than anaerobic exercise (sustained contraction) because blood flow between intermittent contractions would reduce lactate accumulation and provide oxygen for pyruvate metabolism. In contrast to

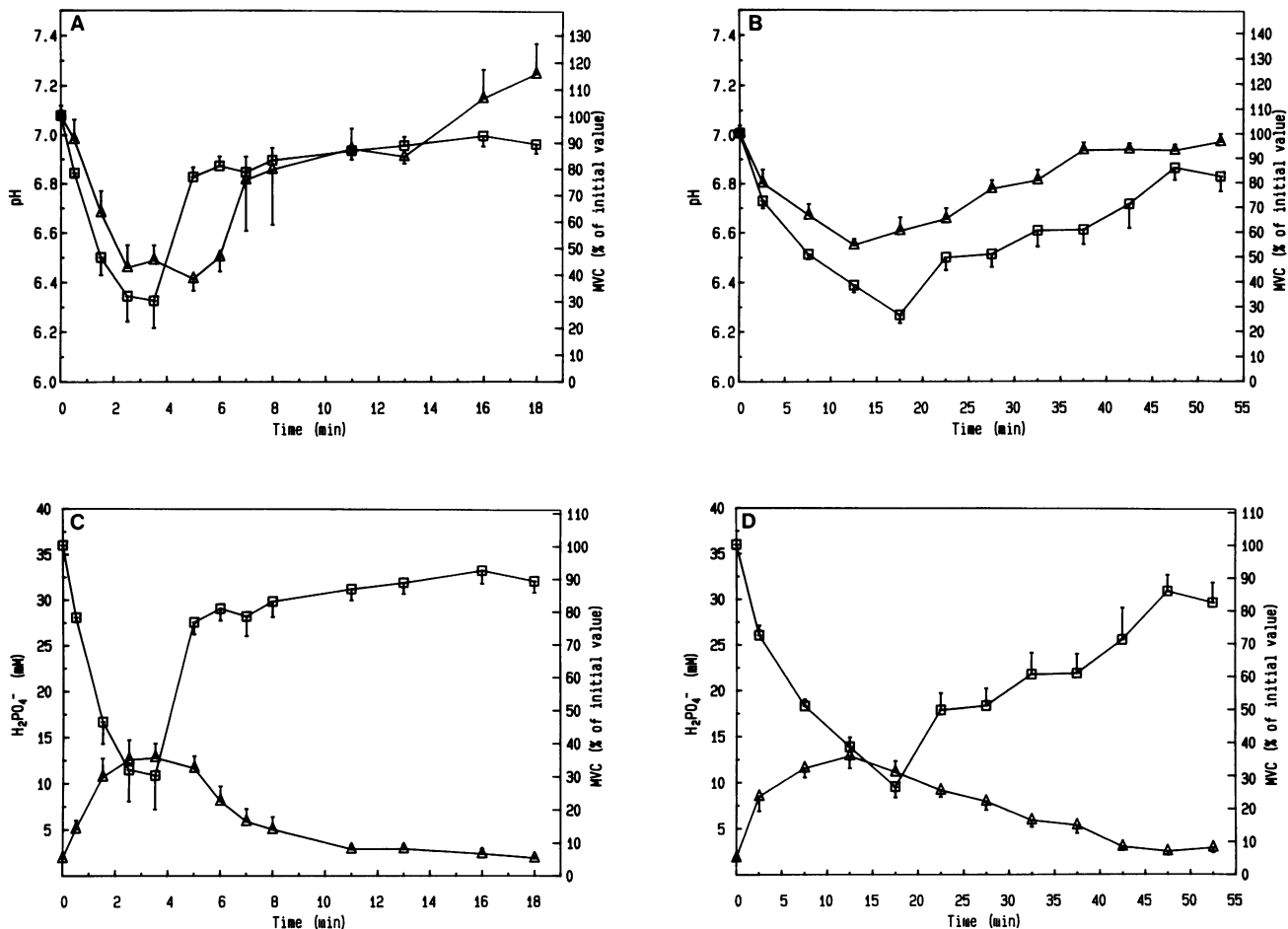


Figure 2. pH (Δ) and MVC (\square) plotted as a function of time during 4 min sustained (A) and intermittent (B) exercise. $H_2PO_4^-$ (Δ) and MVC (\square) plotted as a function of time during 4 min sustained (C) and intermittent (D) exercise.

this expected result, the degree of acidification was almost the same in both intermittent and sustained exercise, at comparable levels of MVC, indicating that the differences in blood flow between the two types of exercise were not sufficient to produce differences in pH.

The second goal of these experiments was to characterize the relationship of metabolic parameters to fatigue in normal human skeletal muscle. The results confirm previous observations that muscle ATP concentrations are stable during both sustained and intermittent exercise. Thus, the large (75%) reduction of MVC cannot be explained by alterations of whole cell ATP. The possibility that diminished ATP concentrations in a small compartment causes fatigue cannot be discounted, although the abundant creatine kinase in muscle should maintain all ATP and PCr in equilibrium. The results also confirm that fatiguing exercise is associated with a fall of PCr and concomitant rise of Pi (24–27). However, the data for both sustained and intermittent contraction clearly show a dissociation between changes in both PCr and Pi and MVC. In both exercise protocols PCr fell more rapidly, and Pi rose more rapidly, than the fall of MVC. This lack of correlation between PCr or Pi and MVC is best illustrated in Figs. 4 and 5, which show that PCr and Pi may change over a wide range with minimal concomitant change in isometric tension. One interpretation of these results is that the changes of Pi and PCr

reflect altered ADP concentrations, which regulate the rates of glycolytic and oxidative metabolism. Nevertheless, despite the important role of these factors as metabolic regulators, the non-linear relationship between these metabolites and MVC indicate that they are not major causative factors in fatigue.

A major finding of the present experiments is that there was a roughly linear correlation between accumulation of H^+ and decline of MVC during fatigue in both types of exercise. A close relationship between accumulation of H^+ and fatigue has been noted since 1807 (quoted by Lehman [9]). There is evidence that low pH diminishes force generation in skinned skeletal muscle of human and rabbit, and effects both slow and fast fibers (8, 31). Sahlin et al. (6, 7) reported that intracellular acidosis produced by high pCO_2 diminished muscle contraction independent of ATP. However, muscle with myophosphorylase and phosphofructokinase deficiency fatigues even more rapidly than normal muscle (24, 32), even though pH does not fall. Furthermore, iodoacetic acid poisoning (which inhibits glycolysis and lactic acid production) does not prevent fatigue in isolated frog muscle (33). On the other hand, changes of pH were not sufficient to account for the decline of force generation in frog muscle (34). These findings suggest that acidosis can not be the only cause of muscle fatigue.

The present results are consistent with a role for H^+ in fatigue but do not necessarily indicate a cause and effect rela-

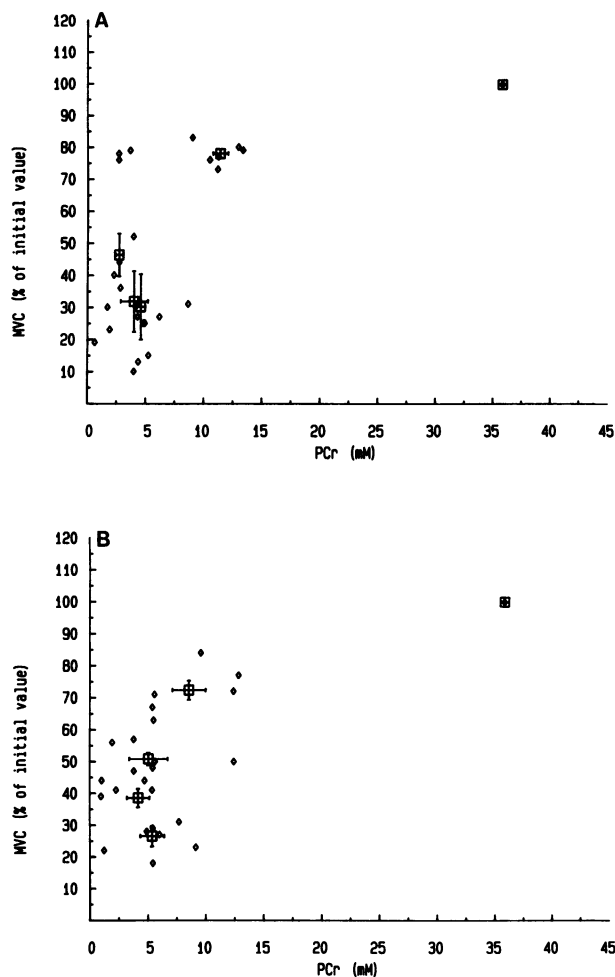


Figure 3. MVC plotted as a function of PCr for sustained (A) and intermittent (B) exercise. All data points are shown (\diamond), as well as mean \pm SE for each time point (\square).

relationship. Even if such a causal relationship is present, the mechanism is uncertain. One possibility is that increased H^+ enhances Ca^{2+} binding to sarcoplasmic reticulum (35), making a higher concentration of Ca^{2+} necessary to generate a given force (36). Accumulation of H^+ may also directly affect the contractile mechanism (discussed above), may alter muscle membrane depolarization (37), or may alter excitation-contraction coupling (38). Although some combination of these events remains a possibility, other data from this laboratory suggest that neither changes in excitation-contraction coupling nor alterations of the muscle membrane parallel changes in pH or MVC, at least during recovery (13).

Dawson et al. (17) originally proposed that $H_2PO_4^-$ may directly inhibit actinomyosin cross-bridge formation; this suggestion was based on ^{31}P NMR studies of amphibian muscle in vitro (18). Nosek et al. (19) recently obtained similar results using skinned rabbit muscle fibers, confirming that $H_2PO_4^-$ may be an important determinant of muscle fatigue. The present results, demonstrating a significant correlation between $H_2PO_4^-$ and MVC, are consistent with a role for $H_2PO_4^-$ in human muscle fatigue. Further experiments are necessary to establish the role of $H_2PO_4^-$, and to ascertain if changes in $H_2PO_4^-$ are responsible for some of the effects previously attrib-

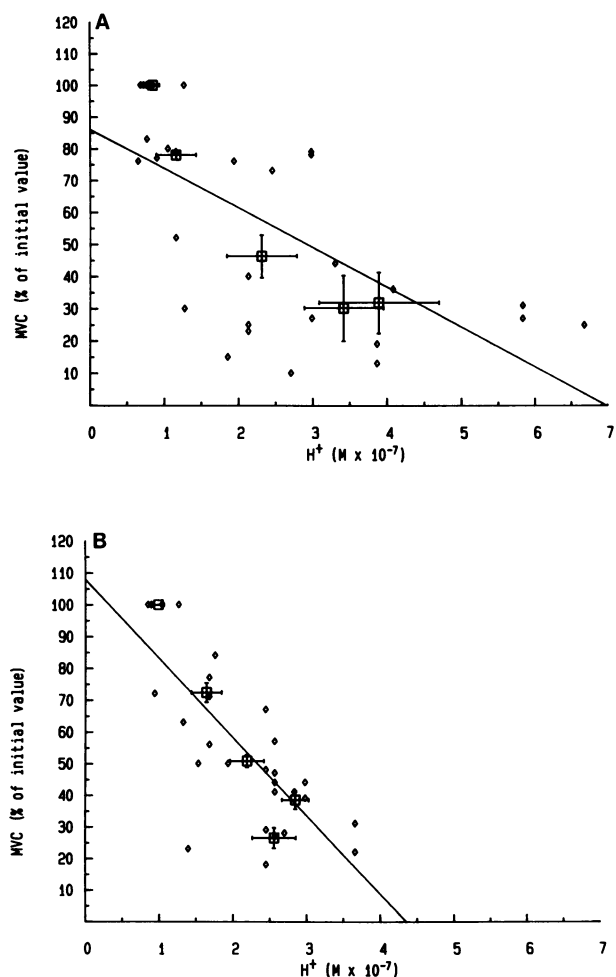


Figure 4. MVC plotted as a function of H^+ for sustained (A) and intermittent (B) exercise. All data points are shown (\diamond), as well as mean \pm SE for each time point (\square). r values are: (a) 0.64; (b) 0.77.

uted to H^+ . Because $H_2PO_4^-$ concentration depends upon both total Pi concentration and pH, alterations of high energy phosphate metabolism as well as changes of carbohydrate (i.e., lactic acid) metabolism will influence $H_2PO_4^-$. This raises the possibilities that $H_2PO_4^-$ may be partially responsible for the fatigue that occurs in metabolic myopathies which are characterized by inhibition of glycolysis (24, 32); in these conditions pH does not fall, but Pi (and thus $H_2PO_4^-$) rapidly rises due to PCr hydrolysis.

The interpretation of the present experiments are complicated for several reasons. First, the changes in MVC might not be due to alterations in muscle contractility, but could be caused by central fatigue. For example, it might be argued that accumulation of H^+ is associated with muscle pain, producing a secondary decrease in central activation of the muscle in question. However, our recent results and previous reports indicate that the contribution of central fatigue is negligible (39, 40). The second problem concerns the fact that the adductor pollicis is composed of $\sim 80\%$ type I and 20% type II fibers which fatigue differently during exercise (41, 42). Changes of MVC and metabolites represent an increase of all fibers detected by the coil. It is possible that a portion of the fibers maintain their force and metabolites, while other fibers

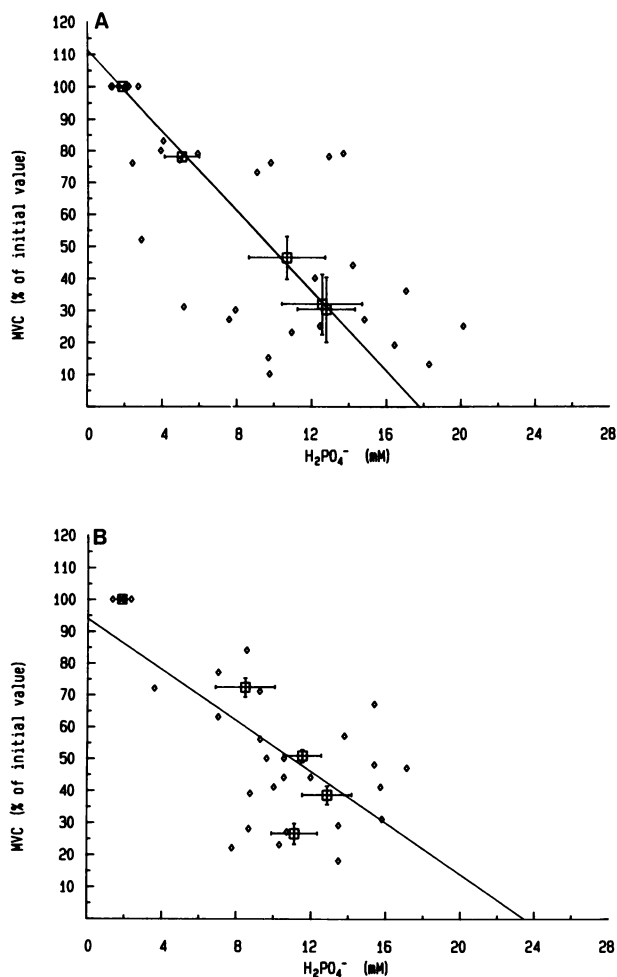


Figure 5. MVC plotted as a function of H_2PO_4^- for sustained (A) and intermittent (B) exercise. All data points are shown (\diamond); as well as mean \pm SE for each time point (\square). r values are: (a) 0.70; (b) 0.73.

exhibit dramatic changes. Further experiments are necessary to investigate the role of fiber type composition in muscle fatigue. Third, the present experiments measured net metabolite levels rather than the unidirectional rates of ATP and PCr exchange. The turnover rates of high energy phosphates can be measured by NMR magnetization transfer techniques (43). Bittle and Ingwall (44) have demonstrated that the turnover rate of creatine kinase is directly related to cardiac work, despite virtually no change in steady state high energy phosphate concentrations. Therefore, magnetization transfer experiments of human muscle might demonstrate a closer relationship between turnover rates and MVC than exists between net concentrations and MVC. Fourth, the low signal/noise of ATP and changes of coil Q due to muscle movement prevent accurate calculation of the total phosphate and total adenine nucleotide pools. Finally, it is recognized that muscular fatigue is a complex and multifactorial process. No single parameter or metabolite is likely to show a close correlation to MVC under all experimental conditions.

In conclusion, the present experiments demonstrate linear relationships between H^+ , H_2PO_4^- , and muscular fatigue. The significance of these findings is underscored by previous reports that under some conditions, acidosis increases and alka-

losis reduces human muscular fatigue (10). Therefore, maneuvers designed to prevent intracellular acidification might lead to decreased muscular fatigue. This therapeutic effect might be useful in sports, physically demanding work, and various diseases. However, before therapeutic measures can be instituted, further studies are necessary to carefully delineate the relationships between fatigue, intracellular pH, and H_2PO_4^- in a variety of circumstances.

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