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ORIGINAL ARTICLE

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Effect of circulatory occlusion on human muscle metabolism during exercise and recovery

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Abstract To assess muscle metabolism and inorganic phosphate (P_i) peak splitting during exercise, 31-phosphorus nuclear magnetic resonance spectroscopy was performed during ramp incremental and submaximal step exercise with and without circulatory occlusion. Seven healthy men performed calf flexion in a superconducting magnet. There was no P_i splitting during ramp incremental exercise with the circulation present and phosphocreatine (PCr) decreased linearly by 0.07 (SEM 0.01) $mmol \cdot l^{-1} \cdot s^{-1}$, while exercise with the circulation occluded caused the P_i peak to split into a high and a low pH peak. The rate of PCr decrease during exercise with the circulation occluded was 0.15 (SEM 0.03) $mmol \cdot l^{-1} \cdot s^{-1}$ which with the efficiency of the adenosine 5′-triphosphate (ATP) hydrolysis reaction corresponded well to the mechanical energy. Both with and without occlusion of the circulation PCr decreased with some time lag which may reflect the consumption of residual oxygen. In submaximal step exercise PCr decreased exponentially at the onset of exercise with the circulation open whereas it decreased linearly by 0.15 $mmol \cdot l^{-1} \cdot s^{-1}$ when the circulation was occluded. After exercise, occlusion of the circulation was maintained for 1 min more and there was no PCr resynthesis. It is suggested that ATP synthesis was limited by the availability of oxygen.

Key words 31-Phosphorus nuclear magnetic resonance spectroscopy · Phosphocreatine · Adenosine 5′-triphosphate · Inorganic phosphate splitting · Circulatory occlusion

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Introduction

31-Phosphorous nuclear magnetic resonance spectroscopy (^{31}P MRS) is used noninvasively to assess concentrations of phosphorous compounds, such as phosphocreatine (PCr), inorganic phosphate (P_i) and adenosine 5′-triphosphate (ATP), as well as in determining the intramuscular pH during and after exercise. The kinetics of PCr during exercise and recovery is used for the evaluation of the biophysics of muscle contraction. It has been suggested that P_i peak splitting during exercise may be due to the difference between oxidative and glycolytic muscle fibres (Park et al. 1987; Achten et al. 1990; Mizuno et al. 1990; Vandenborne et al. 1991; Yoshida and Watari, 1992a,b, 1993a,b,c,d, 1994; Yoshida et al. 1996). Mizuno et al. (1994a) have reported three patterns of P_i peak during exercise and suggested that the type of P_i peak depended on the percentage of oxidative slow twitch (ST) and glycolytic fast twitch (FT) muscle fibres. Thus, the pattern of the P_i peak during exercise may reflect the contractile and metabolic characteristics of the ST and FT fibres.

We hypothesized that oxygen availability would affect the P_i peak splitting pattern during exercise. The ^{31}P MRS was measured during exercise with and without circulatory occlusion to the calf muscles which possess a volume which is sufficient to obtain a high signal to noise ratio.

Methods

Subjects

Seven healthy sedentary men volunteered as subjects. The approval of the Human Ethics Committee of the National Institute for Physiological Science (Okazaki, Japan) and the written consent of each subject was obtained.

Exercise protocol

Experiments were performed using two types of calf muscle exercise: incremental ramp exercise with the intensity increased by 0.35 W every 15 s until the subject was exhausted and submaximal step exercise was performed for 4 min or until exhaustion at 60% of the exercise intensity attained during maximal exercise. Muscle concentric and eccentric contractions were performed without a pause. The lever of a wooden exercise ergometer, which was designed for use inside the bore of a magnet, was fastened to a foot of each subject while he lay in a prone position. The work intensity was determined from the mass lifted by the foot using a pulley, the flexion rate (set at 50 times $\cdot$ min^{-1} following a metronome), and the vertical displacement of the mass. Incremental ramp exercise was performed both with and without the blood flow occluded at 280 mmHg with a tourniquet. In submaximal step exercise, the blood flow was occluded from the beginning of exercise and, in a second series of experiments, after 2 min of exercise. At exhaustion circulatory occlusion was continued for 1 min more and then recovery was followed.

^{31}P MRS acquisition

The ^{31}P MRS spectra were obtained using a 67-cm bore 2.1-T superconducting magnet (Hitachi, Japan) and a spectrometer (EX90, Jeol, Japan). The subject's body was placed inside the bore of the magnet and a surface coil (8 cm in diameter) was placed over the centre of the calf muscle to obtain a ^{31}P MRS signal. After the subject was placed in the magnet, magnetic field homogeneity was optimized by shimming on a proton signal of the calf muscle. The ^{31}P MRS data were collected with an optimal pulse width at a pulse rate of one per 0.416 s. The ^{31}P MRS data for 12 scans were averaged to produce a single spectrum, so each represented the data from a 5.0-s period.

Data analysis

Relative areas under the P_i and PCr peaks of the spectrum were determined by integration. Intramuscular pH was determined using the chemical shift of P_i relative to PCr, pH = 6.73 + log10{(a − 3.275) / (5.685 − a)}, where a is the chemical shift from P_i to PCr. When the P_i peak was split, the P_i peaks were separated by a model fitting procedure using the Lorentzen curve which employs the least mean squares method (Yoshida and Watari 1994).

Statistical analysis

Differences between mean values obtained for exercise and recovery periods were compared by Student's t-test. A probability value of less than 0.05 was considered significant.

Results

Calf muscle ^{31}P MRS spectra were obtained with the intensity increased by 0.35 W in every 5 s until the subject was exhausted. The ^{31}P MRS of the calf muscle at rest, during incremental exercise and recovery is demonstrated as stacked plots with and without the circulation occluded for one person in Fig. 1. No P_i peak splitting was observed with the circulation open but splitting was seen during exercise with the circulation occluded that is, the P_i peak split into a high pH P_i peak and a low-pH P_i peak. The high pH P_i peak was maintained at the same value throughout the exercise, while the low pH P_i peak decreased down to pH 6.2. Similar data were obtained from the other volunteers.

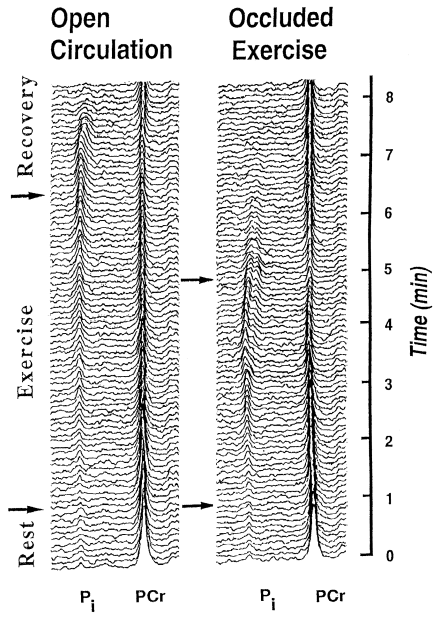

Fig. 1 One example of ^{31}P magnetic resonance spectra of human calf muscle during rest, exercise and recovery periods. The exercise increased by 0.35 W every 15 s until exhaustion. The figure shows results with an open circulation (*left panel*) and occluded blood flow at 280 mmHg (*right panel*). In these figures only signals for inorganic phosphate (P_i) and creatine phosphate (*PCr*) are shown. The arrow (→) indicates the time of the start of exercise and of exhaustion

Figure 2 shows the time course of PCr, which has been averaged for seven persons, with and without circulatory occlusion. During incremental exercise, PCr decreased linearly by 0.07 (SEM 0.01) mmol $\cdot$ l^{-1} $\cdot$ s^{-1},

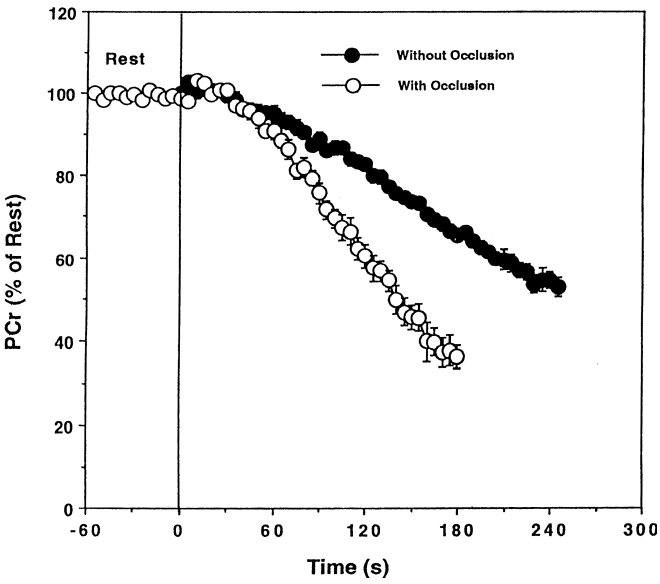

Fig. 2 Averaged time courses of creatine phosphate (*PCr*) during ramp incremental exercise until exhaustion with and without circulatory occlusion. During incremental exercise *PCr* decreased linearly by 0.07 (SEM 0.01) mmol $\cdot$ l^{-1} $\cdot$ s^{-1}, while the rate during exercise with the circulation occluded was 0.15 (SEM 0.03) mmol $\cdot$ l^{-1} $\cdot$ s^{-1}

while the rate during exercise with the circulation occluded was by 0.15 (SEM 0.03) $mmol \cdot l^{-1} \cdot s^{-1}$.

The behaviour of PCr for one volunteer at rest and during submaximal step exercise and recovery is shown in Fig. 3. Two more experiments were made with the same person after allowing time for recovery:

1. Blood flow was restricted from the beginning of exercise with a tourniquet applied at 280 mmHg until exhaustion, and
2. Also 2 min after the start of the exercise.

The PCr decreased linearly in these two experiments at the same rate as in the initial stage of the experiment with the circulation open. The rate of PCr decrease was $0.15\,mmol \cdot l^{-1} \cdot s^{-1}$ during circulatory occlusion and was manifested in all the volunteers. The data for PCr resynthesis were normalized and the time constant was found to be 33.4 (SEM 5.1) s.

The ratio of P_i to PCr (P_i:PCr ratio) with and without circulatory occlusion in the ramp incremental exercise was calculated from the experiments of Fig. 2, as shown in Fig. 4 (top). In the submaximal step exercise the ratios were also calculated from the experiments of Fig. 3, as shown in Fig. 4 (bottom).

When circulatory occlusion continued 1 min more after exhaustion following submaximal step exercise with the circulation occluded no PCr resynthesis was observed (Fig. 5). This procedure was compared with the experiment in which occlusion was released immediately after the submaximal step exercise.

Discussion

P_i peak splitting during exercise

The significance of P_i peak splitting during exercise of human muscle has been debated. The splitting has been considered to be due to data being acquired from active and inactive muscle groups, because the detection of splitting depends on the size of the surface coil (Taylar et al. 1983; Jeneson et al. 1989). Alternatively, P_i peak splitting may be due to a physiological difference between ST and FT fibres. The possibility of the P_i peak splitting being due to differences between the intracellular and interstitial compartments and to muscle fibres performing different degrees of work has also been considered. Mizuno et al. (1994a) have reported three patterns of P_i peak during exercise and suggested that the type of P_i peak depends on the involvement of ST and FT fibres. Mizuno et al. (1994b) have also demonstrated that the splitting of the P_i peak reflects metabolic differences between oxidative and glycolytic fibres of skeletal muscle by using the effect of a depolarizing and a nondepolarizing neuromuscular blocking agent on the ^{31}P MRS during forearm exercise.

Since differences in the contractile and metabolic characteristics of ST and FT fibres alter the pattern of muscle fibre recruitment during exercise, the P_i peak pattern may reflect these differences. Although P_i peak splitting during exercise has been observed in handgrip exercise and knee flexion, there was no P_i splitting during calf muscle exercise in these sedentary subjects and P_i peak was also maintained at rest values as shown in Fig. 1, left panel. This suggests that the FT fibres were active but at a low level during incremental exercise with an open circulation except just before exhaustion when pH changed.

During exercise with the circulation occluded P_i peak splitting was observed, as shown in Fig. 1, right panel. The high pH peak was at the same pH as at rest, and after an initial increase of P_i, the signal intensity was maintained at a constant value except for the end period which was affected by exhaustion. The low pH peak appeared abruptly from the original P_i peak 2 min after the start of exercise when the acidic lactate concentration would have overcome the buffer action at the inside of the sarcosome. Then the pH decreased gradually to 6.2 with an increasing intensity of P_i. This suggests that the oxygen remaining in the ST muscle fibre was utilized thus showing little increase in the high pH peak and that the FT fibre was active to the full from the beginning of exercise and producing lactate.

PCr hydrolysis during circulatory occlusion

It has been shown that PCr hydrolysis is controlled by myosin ATPase and sarcomere creatine kinase, and PCr

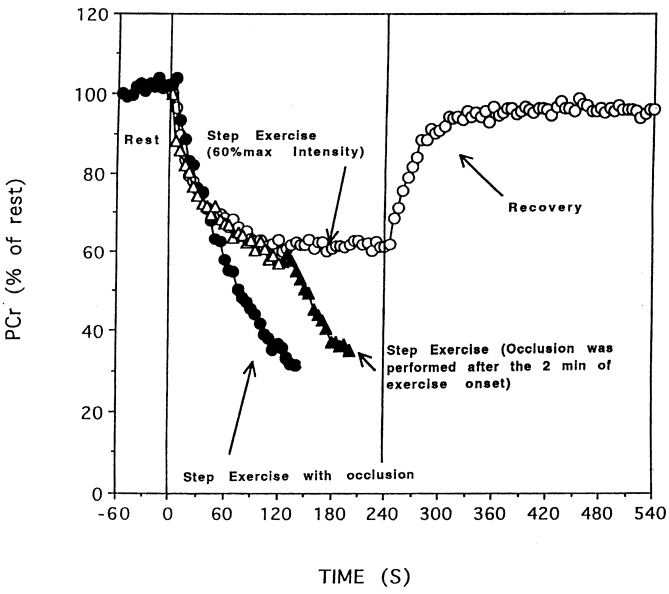

Fig. 3 One example of creatine phosphate (*PCr*) behaviour during submaximal step exercise and recovery (○). Results from additional experiments are also shown: blood flow restricted with a tourniquet at 280 mmHg from the beginning of exercise (●) and 2 min later during the exercise (△ and ▲). In these two procedures *PCr* decreased linearly at the same rate until exhaustion. In the recovery process after exercise with the circulation open the time constant of an increase of *PCr* was calculated as of 33.4 (SEM 5.1) s

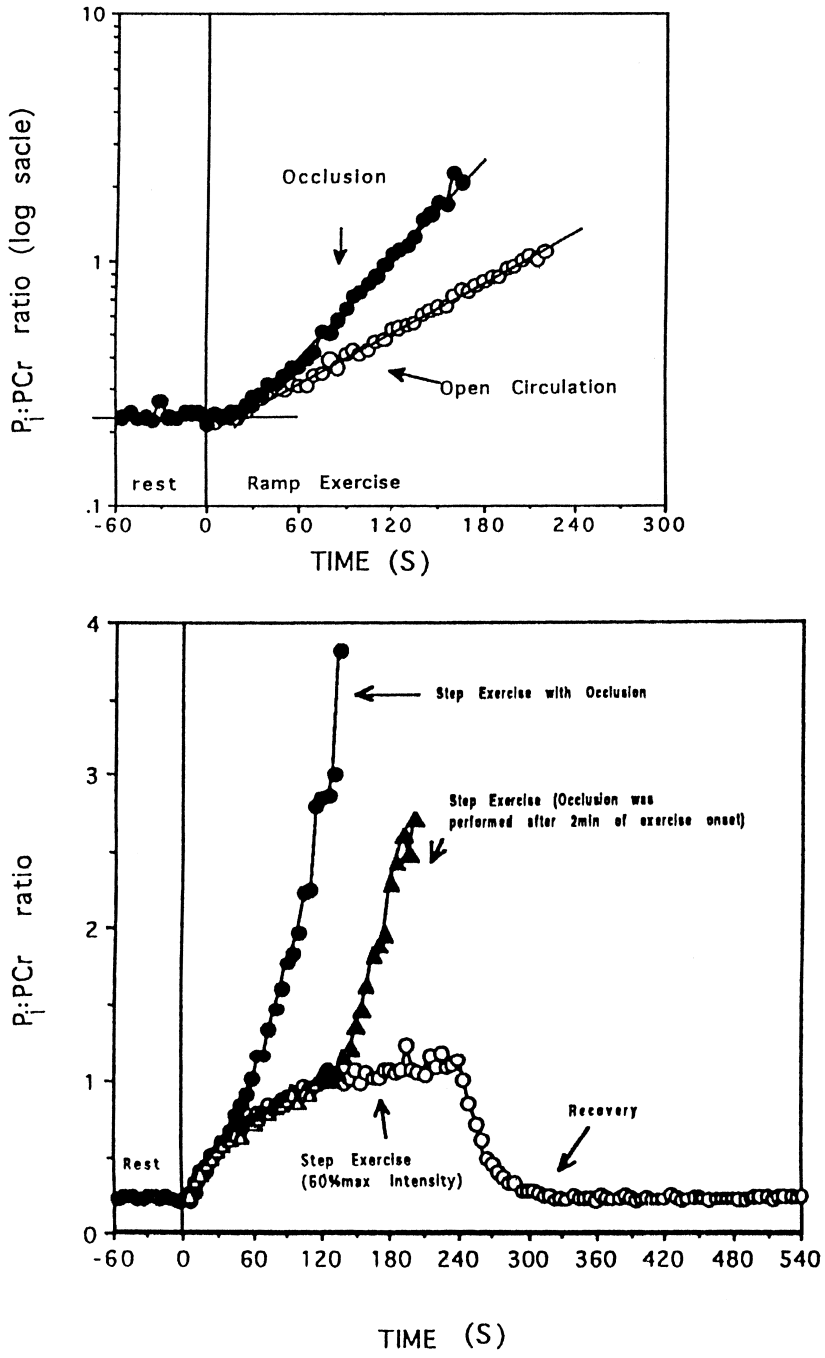

Fig. 4 The ratio of inorganic phosphate to creatine phosphate (P_i:*PCr* ratio) with and without circulatory occlusion. The ratios in ramp incremental exercise (*top*) were obtained from the experiments as shown in Fig. 2. In the submaximal step function exercise (*bottom*) the ratios were calculated from the experiment as shown in Fig. 3

resynthesis during recovery is regulated by aerobic metabolism and mitochondrial creatine kinase (Mole et al. 1985). During exercise, the rate of PCr decrease might be influenced by the imbalance between PCr hydrolysis at the myofibril and simultaneous synthesis in the mitochondria. As shown in Fig. 2, there was a linear decrease in PCr in both cases, especially in the experiment with the circulation occluded, characteristic of a time lag. This time lag was derived from the residue of the oxidative energy source and furthermore was comparable with that in the condition where the occlusion was delayed. With and without circulatory occlusion the mean PCr degradation rates were calculated as $0.15\,\mathrm{mmol}\cdot\mathrm{l}^{-1}\cdot\mathrm{s}^{-1}$ and $0.07\,\mathrm{mmol}\cdot\mathrm{l}^{-1}\cdot\mathrm{s}^{-1}$, respectively. The difference of the rates is clearly due to lack of oxygen supply and in this situation an oxidative supplement of PCr would no longer be expected. Using the PCr decrease rate measured in incremental exercise with the circulation occluded, the energy expended could be calculated. Supposing the efficiency of the ATP hydrolysis reaction to be 50%, the chemical energy consumed corresponded quite well with the value for mechanical energy.

The PCr was also measured during the submaximal step exercise and recovery with the circulation open. Two more experiments were done at a different time but for the same subject: blood flow restriction from the beginning of exercise with a tourniquet at 280 mmHg until exhaustion, and that at 2 min after the start of exercise, as shown in Fig. 3. The PCr decreased linearly at the same rate for these experiments during circulatory occlusion and the rate of PCr decrease was determined as $0.15\,mmol \cdot l^{-1} \cdot s^{-1}$. These phenomena were observed in every volunteer.

At exhaustion in the incremental experiments the PCr contents remained, and were more prominent in the exercise without occlusion of the circulation. When a Langendorf preparation of the frog heart is used to measure phosphorous compounds by ^{31}P MRS in anaerobic conditions PCr has been shown to be consumed completely and even ATP to be completely depleted (see Takami et al. 1988). The time of exhaustion was left to the subject to decide and the experiment with occlusion of the circulation was more taxing than that without occlusion. The period to exhaustion was therefore another feature of PCr content.

P_i:PCr ratio during exercise with the circulation occluded

According to Chance et al. (1986), regulation of phosphorylation is analysed in terms of enzyme kinetics as a function of substrate concentrations with a sufficient oxygen supply. It is considered adenosine 5′-diphosphate is a regulator and is suggested to be proportional to the P_i:PCr ratio. However, from enzyme kinetics there is no information on anaerobic glycolytic reactions. The ratio has been used as an estimate of phosphorylation potential (March et al. 1991). In Fig. 4 P_i:PCr ratios were seen to be increased more promptly during exercise with the circulation occluded than with the circulation open so it would be due to a reduced or no oxygen availability. If it is supposed that, in the system, a PCr of $25\,mmol \cdot l^{-1}$, P_i of $5\,mmol \cdot l^{-1}$, creatine of $13\,mmol \cdot l^{-1}$, ATP of $5\,mmol \cdot l^{-1}$ and ADP of $0.0016\,mmol \cdot l^{-1}$ exist at rest, the overall equilibrium constant of coupled reactions of mitochondrial and myosin ATPase, and mitochondrial and sarcosomere creatine kinase may be calculated as 162. Assuming that the sum of creatine and PCr, and of PCr and P_i are constant, and that there is no net hydrogen ion generation, it may be easily derived that the ratio calculated at rest is 0.2, and after exercise is 0.5 at 20% hydrolysis of PCr, as 1.0 at 40%, 2.0 at 60% and as 5.0 at 80%. Therefore the high ratios at PCr hydrolysis in exercise with the circulation occluded were due to a contribution of glycolytic reactions in muscle fibres where hydrogen ions are generated in an overall reaction. Actually a low pH peak appeared in exercise with the circulation occluded with a high P_i:PCr ratio.

PCr resynthesis rate after exercise with the circulation occluded

After exercise, the P_i was quickly used to synthesize ATP via mitochondrial oxidative phosphorylation and PCr was synthesized accordingly. If aerobic metabolism was inhibited by circulatory occlusion during recovery, PCr resynthesis would be not expected. In this context, the PCr recovery data could be used to estimate the rate of oxidative metabolism. It has been reported that the rate of PCr resynthesis after exercise may be greater in ST than in FT fibres (Tesch et al. 1989).

In the present study, the PCr resynthesis rate was inhibited after circulatory occlusion (Fig. 5). Upon restoration of blood flow PCr recovery followed an exponential function with a time constant of PCr resynthesis of 44.5 (SEM 9.2) s, and was slower than that after the exercise with the circulation open at a time constant of 33.4 (SEM 5.1) s. Granting that ATP production in both cases was the same, the influx of sodium ions and efflux of potasium ions during exercise with the circulation occluded were possibly larger than when the circulation was open so the ATP produced was used for resynthesis of PCr as well as the recovery to normal ion gradients in the muscle cell.

When maintaining occlusion for 1 min more after exercise with the circulation occluded PCr recovery could not be observed, as shown also in Fig. 5. This means either that resynthesis of PCr could not begin even in the recovery process, or that the system was in quasi-equilibrium with no oxygen. This phenomenon

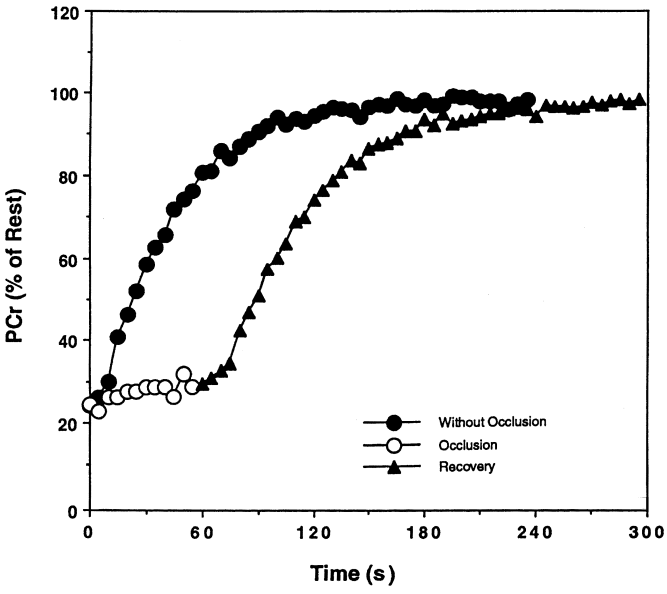

Fig. 5 Creatine phosphate (*PCr*) resynthesis with the circulation open after the submaximal step exercise with the circulation occluded until exhaustion (●) and after the same exercise and 1 min more continuing circulatory occlusion without exercise (○ and ▲). In the latter case no *PCr* resynthesis was observed until removal of occlusion when the recovery started. The rate of resynthesis in both cases was the same with a time constant of 44.5 (SEM 9.2) s

might be understood to derive from a lack of nutrients, such as glucose, due to obstruction of the blood flow. This phenomenon has been reported by Quistroff et al. (1992).

Conclusion

In conclusion, ^{31}P MRS was performed during ramp incremental exercise of calf flexion with the circulation open, when there was no P_i splitting and PCr decreased linearly; during exercise with the circulation occluded the P_i peak was split into a high and a low pH peak. The appearance of the additional low pH P_i peak was suggested as FT muscle fibre recruitment. The rate of PCr decrease during exercise with the circulation occluded was $0.15 mmol \cdot l^{-1} \cdot s^{-1}$ which might correspond to the ATP synthesis rate. After exhaustion as a result of exercise, occlusion was maintained for a further 1 min and no PCr resynthesis was observed. It is suggested that the ATP synthesis was limited by the lack of oxygen.

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References

Achten E, Van Cauteren M, Willem R, Luypaert R, Malaisse WJ, Van Bosch G, Delanghe G, De Meirleir K, Osteaux M (1990) ^{31}P-NMR spectroscopy and the metabolic properties of different muscle fibre. J Appl Physiol 68:644–649

Chance B, Leigh JS Jr, Kent J, McCully K (1986) Metabolic control principles and ^{31}P-NMR. Fed Proc 45:2915–2920

Jeneson JAL, Wesseling MW, De Boer RW, Amelink HG (1989) Peak-splitting of inorganic phosphate during exercise: anatomy or physiology? A MRI-guided ^{31}P MRS study of human forearm muscle. In: Proceedings of the Eighth Annual Meeting of the Society of Magnetic Resonance in Medicine, Society of Magnetic Resonance in Medicine, Berkeley, Calif.

March GD, Paterson DH, Thompson RT, Driedger AA (1991) Coincident thresholds in intracellular phosphorylation potential and pH during progressive exercise. J Appl Physiol 71:1076–1081

Mizuno M. Justesen LO, Bedolla J. Friedman DB, Secher NH, Quistorff B (1990) Partial curarization abolishes splitting of the inorganic phospate peak in ^{31}P-NMR spectroscopy during intense forearm exercise in man. Act Physiol Scand 139:611–612

Mizuno M, Seeher NH, Quistroff B (1994a) ^{31}P-NMR spectroscopy. rsEMG, and histochemical fibre types human wrist flexor muscles. J Appl Physiol 76:531–538

Mizuno M, Horn A, Seeher NH, Quistroff B (1994b) The exercise-induced ^{31}P-NMR metabolic response of human wrist flexor muscles during partial neuromuscular blockade. Am J Physiol 267 R408–R414

Mole PA, Coulson RL, Caton JR, Nicoles BG, Barstow TJ (1985) In vivo ^{31}P-NMR in human muscle: transient patterns with exercise. J Appl Physiol 59:101–104

Park JH, Brown RL, Park CR, McCully KK, Cohn M, Hasellgrove J, Chance B (1987) Functional pools of oxidative and glycolytic fibers in human muscle observed by ^{31}P magnetic resonance spectroscopy during exercise. Proc Natl Acad Sci USA 84:8976–8980

Quistroff B, Johansen L, Sahlin K (1992) Absence of phosphocreatine resynthesis in human calf muscle during ischemic recovery. Biochem J 291:681–686

Takami H, Furuya E, Tagawa K, Seo Y, Murakami M, Watari H, Matsuda H, Hirose H, Kawashima Y (1988) NMR-invisible ATP in rat heart and its change in ischemia. J Biochem 104:35–39

Taylar DJ, Bore PJ, Styles P, Gadian DG, Radda GK (1983) Bioenergetics of intact human muscle. A ^{31}P -nuclear magnetic resonance study. Mol Biol Med 1:77–94

Tesch PA, Thorsson A, Fujitsuka N (1989) Creatine phosphate in fibre types of skeletal muscle before and after exhaustive exercise. J Appl Physiol 66:1756–1759

Vandenborne K, McCully K, Kakihara H, Prammer M, Bolinger L, Detre JA, De Meirleire K, Walter G, Chance B, Leigh JS Jr (1991) Metabolic heterogeneity in human calf muscle during maximal exercise. Proc Natl Acad Sci USA 88:5714–5718

Yoshida T, Watari H (1992a) Noninvasive and continuous determination of energy metabolism during muscular contraction and recovery. Med Sport Sci 37:364–373

Yoshida T, Watari H (1992b) Muscle metabolism during repeated exercise studied by ^{31}P-MRS. Ann Physiol Anthropol 37:364–373

Yoshida T, Watari H (1993a) ^{31}P -nuclear magnetic resonance spectroscopy study of the time course of energy metabolism during exercise and recovery. Eur J Appl Physiol 66:494–499

Yoshida T, Watari H (1993b) Metabolic consequence of repeated exercise in long distance runners. Eur J Appl Physiol 67:261–265

Yoshida T, Watari H (1993c) Changes in intracellular pH during repeated exercise. Eur J Appl Physiol 67:274–278

Yoshida T, Watari H (1994) Exercise-induced splitting of the inorganic phosphate peak: investigation by time-resolved ^{31}P -nuclear magnetic resonance spectroscopy. Eur J Appl Physiol 69:465–473

Yoshida T, Watari H Tagawa K (1996) Effects of active and passive recoveries on splitting of the inorganic phosphate peak determined by ^{31}P -nuclear magnetic resonance spectroscopy. Nucl Mag Reson Biomed 9:13–19