REVIEW ARTICLE

Sports Med 2002; 32 (12): 761-784
0112-1642/02/0012-0761/$25.00/0

Factors Affecting the Rate of Phosphocreatine Resynthesis Following Intense Exercise

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Abstract

Within the skeletal muscle cell at the onset of muscular contraction, phosphocreatine (PCr) represents the most immediate reserve for the rephosphorylation of adenosine triphosphate (ATP). As a result, its concentration can be reduced to less than 30% of resting levels during intense exercise. As a fall in the level of PCr appears to adversely affect muscle contraction, and therefore power output in a subsequent bout, maximising the rate of PCr resynthesis during a brief recovery period will be of benefit to an athlete involved in activities which demand intermittent exercise. Although this resynthesis process simply involves the rephosphorylation of creatine by aerobically produced ATP (with the release of protons), it has both a fast and slow component, each proceeding at a rate that is controlled by different components of the creatine kinase equilibrium. The initial fast phase appears to proceed at a rate independent of muscle pH. Instead, its rate appears to be controlled by adenosine diphosphate (ADP) levels; either directly through its free cytosolic concentration, or indirectly, through its effect on the free energy of ATP hydrolysis. Once this fast phase of recovery is complete, there is a secondary slower phase that appears almost certainly rate-dependant on the

return of the muscle cell to homeostatic intracellular pH. Given the importance of oxidative phosphorylation in this resynthesis process, those individuals with an elevated aerobic power should be able to resynthesise PCr at a more rapid rate than their sedentary counterparts. However, results from studies that have used phosphorus nuclear magnetic resonance (^{31}P-NMR) spectroscopy, have been somewhat inconsistent with respect to the relationship between aerobic power and PCr recovery following intense exercise. Because of the methodological constraints that appear to have limited a number of these studies, further research in this area is warranted.

1. Energy Production at the Onset of Muscular Contraction

Energy for human activity is derived from the hydrolysis of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) and inorganic phosphate (P_i) as shown in equation 1.

$$ATP^{4-} + H_2O \rightarrow ATPase \rightarrow ADP^{3-} + P_i^{2-} + H^+ + Energy$$

Skeletal muscle cells then transform this chemical energy into mechanical energy for movement. However, despite some studies reporting a significant reduction in ATP concentration ([ATP]),[1,2] this change is usually either small[3,4] or statistically nonexistent.[5,6] This indicates that ATP is being almost entirely regenerated during exercise. Three energy systems contribute to the resynthesis of ATP via ADP rephosphorylation; the relative contribution of these energy systems to ATP resynthesis has been shown to depend upon the intensity of exercise.

Two of the energy systems have been termed 'anaerobic' as they can proceed without oxygen (O_2); these systems are particularly important at the onset of exercise and when the demand for energy is high. Phosphocreatine (PCr), which undergoes breakdown in the reaction shown in equation 2, represents the most immediate energy reserve in skeletal muscle for ATP resynthesis at the onset of muscular contraction.

$$PCr + MgADP^- + H^+ \xleftrightarrow{\text{creatine kinase}} MgATP^{2-} + \text{creatine}$$

Skeletal muscle PCr reserves exceed those of ATP; moreover the catalytic capacity of creatine kinase (CK) is several fold higher than the maximal rate of ATP hydrolysis in working muscle.[7,8] PCr breakdown therefore provides a buffer to potential decreases in [ATP] during exercise. In addition, as PCr rephosphorylates ADP, ATP will be re-utilised for contraction, and this leads to an increased concentration of P_i ([P_i]), which is a substrate and activator of the key glycolytic enzyme phosphofructokinase (PFK). Because of its significant contribution to the energy yield at the onset of near maximal exercise, the concentration of PCr ([PCr]) can be reduced to less than 40% of resting levels within 10 seconds of the start of intense exercise.[9]

The second anaerobic energy system relies upon a series of nine different chemical reactions and is therefore slower to become fully active. However, this particular system (glycogenolysis) is also activated at the onset of muscular contraction.[10,11] Glycogenolysis has a greater capacity to provide energy than PCr, and so it continues to rephosphorylate ADP during maximal exercise after PCr reserves have become essentially depleted. However, during very high-intensity exercise, glycolysis is self-regulating, as the hydrogen ions that dissociate from the glycogenolytic end-product lactic acid can potentially impair contraction. Although there exists a number of possible mechanisms for this action, inhibition of key enzymes and interference with contractile proteins in muscle appear two of the significant consequences of excessive H^+ production in active muscle.[12-15]

Unlike the two energy systems briefly reviewed above, the remaining energy system requires O_2 and is consequently termed the 'aerobic' system. While being unable to produce ATP at an equivalent rate to that produced by PCr breakdown and glycogenolysis, this system is capable of sustaining low-intensity exercise for several hours. However, because of an increased complexity, the time between the onset of exercise and when this system is operating at its full potential is around 45 seconds.[16]

2. Metabolic Consequences of Intense Contraction and Muscle Fatigue

2.1 Introduction

One of the metabolic consequences of high-intensity, short-duration muscle contraction is impaired subsequent performance.[15] While factors such as substrate depletion and thermal stress may strongly contribute to fatigue during prolonged exercise,[17] the precise mechanisms responsible for fatigue during brief high-intensity exercise remain contentious.[18] There is evidence supporting both a reduction in [PCr] caused by the rephosphorylation of ADP,[2,4] as well as the increase in H^+ concentration ($[H^+]$)[19,20] that results from the dissociation of lactic acid, as being two principal mechanisms of action.

2.2 Depletion of Phosphocreatine (PCr)

There is a strong relationship between the decline in force and the decline in [PCr] during high-intensity exercise.[4,21,22] Indeed Casey et al.[2] reported that performance during a subsequent bout of maximal exercise was more closely related to the resynthesis of [PCr] during the preceding recovery interval than to the level of lactate accumulation. The findings of Bogdanis et al.[4] are consistent with earlier studies and imply a causal relationship between fatigue and [PCr].

Sargeant and Dolan,[23] in a series of experiments which examined the effect of prior fatigue induced by submaximal exercise on short-term power output during isokinetic cycling, reported that the recovery of power had a half-time ($t_{½}$) of 32 seconds. This is similar to the $t_{½}$ of PCr resynthesis reported by Petersen and Cooke,[24] and Cooke et al.[25] following exhaustive exercise and provides further support for a causal relationship between these two parameters in response to high-intensity exercise. A similar time-course in both the recovery of [PCr] following exercise and the recovery of peak power during sprint exercise has also been reported.[26,27]

2.3 Decline in Intracellular pH (pH_i)

While it has been shown that lactate itself is unlikely to be a major cause of fatigue,[28] many studies have shown that intramuscular acidosis, as measured by an increase in $[H^+]$, is significantly related to the loss of force and tetanic tension.[12,29] However, the exact mechanism of how a decrease in intracellular pH (pH_i) impairs the contractile process, and hence exercise performance, remains unclear.

Donaldson,[30] used peeled mammalian skeletal muscle fibres to examine the effects of a decrease in pH_i on force production and noted that slow oxidative (type I), slow oxidative glycolytic (type IIa) and fast glycolytic (type IIb) fibres lost 12, 25 and 44% of maximum force respectively as a result of intracellular acidosis. Because of the methodology employed, some possible effects of an increased $[H^+]$ on the contraction process were eliminated, and it was suggested that the increase in $[H^+]$ impaired force generation through end-product inhibition of ATP hydrolysis (equation 3):

$$MgATP^{2-} + H_2O \xrightarrow{ATPase} MgADP^{-} + P_i^{2-} + H^+$$

Studies which have employed the skinned (peeled) fibre technique have also consistently demonstrated an acidosis-induced depression of tension in both skeletal and cardiac muscle.[31,32] While a number of other possible derogatory mechanisms exist, the effect of calcium ion (Ca^{2+}) release from the sarcoplasmic reticulum (SR) is believed to be sensitive to changes in pH_i. Re-

search has shown that a decrease in pH_i from 7.0 to 6.2 will shift the force-pCa^{2+} (–log Ca^{2+} concentration) curve to the right. Consequently, not only is a higher free calcium ion concentration ($[Ca^{2+}]$) needed to achieve a given tension but also an increased $[Ca^{2+}]$ will be required to initiate a contraction.[32,33] While contradictory results can be found regarding whether an acidic intracellular environment inhibits the affinity of Ca^{2+} for troponin,[34,35] the observation that low pH_i decreases force even when Ca^{2+} is at saturating concentrations,[33] suggests that interference with the binding of Ca^{2+} to troponin is not the only influencing factor relating pH_i to fatigue.

An exercise-induced fall in pH_i may also impair the capacity for glycogenolysis to provide energy for ATP resynthesis. Hill[36] observed that production of lactate ceased when the pH of electrically stimulated muscle reached 6.3. It has also been suggested that a rise in $[H^+]$ inhibits the activity of the regulatory glycolytic enzyme PFK;[37] inhibition of PFK has been shown to increase the concentrations of fructose-6-phosphate (F-6-P) and glucose-6-phosphate (G-6-P), which may in turn inhibit both hexokinase and phosphorylase. This may explain results reported following three 30-second bouts of sprint cycling between which muscle was sampled using needle biopsy.[15] Observed decreases in glyceraldehyde-3-phosphate and pyruvate following the third bout of maximal exercise implicated a reduced glycolytic flux. It was suggested that as the demand for ATP was still maximal, a likely cause of the reduced glycolytic flux was inhibition of glycolysis as a result of the high $[H^+]$.[15]

Sahlin et al.[18] have proposed a theory that the negative effect of acidosis on muscle contraction is an 'indirect' one that operates through impairment of the ATP generating process (rather that at the contractile proteins). It is proposed that the effect of acidosis on performance is mediated through impaired ATP resynthesis.[18] Some have suggested this to be unlikely, since [ATP] rarely falls below 70% of resting levels even at fatigue[2,6] and therefore remains well above the Michaelis constant (K_m) for myosin adenosine tri-phosphatase (ATPase).[38] However, ATP in the cell may be compartmentalised; total [ATP] may not therefore reflect the [ATP] available for contraction. In considering the effects of acidosis on contraction, Fitts[39] has suggested that any inhibition of H^+ on the activities of phosphorylase and PFK is likely to be countered by an increase in the concentration of P_i, adenosine monophosphate (AMP) and inosine monophosphate (IMP), which are all known to be potent activators of these two enzymes.[37,40]

To summarise the literature to this point, the depletion of PCr and the reduced pH_i that accompany intense exercise are two factors known to contribute to fatigue. Consequently, an important component of the recovery phase during intermittent high-intensity exercise, is the muscle's ability to return to a state as close to homeostasis as possible, so as to maintain subsequent exercise performance. As PCr resynthesis involves the release of H^+ (equation 2), the recovery of these two components is closely related.

3. Phosphorus Nuclear Magnetic Resonance Spectroscopy (^{31}P-NMR)

Prior to an examination of the mechanisms that determine the rate of PCr resynthesis following intense exercise, it is relevant to briefly discuss some basic fundamentals of phosphorus nuclear magnetic resonance spectroscopy (^{31}P-NMR) as it is becoming an increasingly important technique in the study of muscle metabolism.[41-44]

A ^{31}P-NMR unit utilises a surface coil that is in contact with the muscle under investigation and which emits a radio pulse at a specific frequency that is absorbed into the phosphorus-containing compounds within the muscle. This surface coil also receives the radio frequency pulse when it is re-emitted by these phosphorus-containing metabolites and conveys a spectrum based on the metabolic state of the muscle. Each metabolite has a position in the observed spectrum with the area under the peak at each point corresponding to its relative concentration. In normal skeletal muscle,

peaks exist for the three types of ATP (δ, α and β), PCr and P_i. It is relatively easy therefore, to determine the concentration of any of these metabolites relative to their resting levels.

The ^{31}P-NMR spectra is usually free from peaks representing ADP and AMP for two reasons. As a significant portion of these two compounds are bound to protein, and ^{31}P-NMR can only detect metabolites that are freely in solution or in rapid exchange with the free pool, these compounds will be invisible. Making this point essentially irrelevant, however, is the fact that the concentrations of phosphorus-containing metabolites need to be >1 mmol/L (i.e. higher than the surrounding signal noise) if they are to be detected by ^{31}P-NMR.

In addition to allowing calculation of the relative concentrations of metabolites, ^{31}P-NMR also allows the absolute level of many of these metabolites to be determined. However, this calculation requires some assumptions to be made. It is usually assumed that: (i) [ATP] in skeletal muscle is 8.2 mmol/L of intracellular water; (ii) the concentration of total creatine is 42.5 mmol/L of intracellular water; (iii) PCr + P_i equals 42.2 mmol/L of intracellular water; (iv) these compounds are uniformly distributed throughout the approximate 670cm^3 of intracellular water; and that (v) there is no change in total creatine during exercise.[45] In addition, the equilibrium constant (K_{eq}) for the CK reaction (equation 2) is assumed to have a value of approximately 1.66×10^9 mol^{-1}[46] when calculated at a pH of 7, a temperature of 38°C, an ionic strength of 0.25 and assuming 1 mmol of free Mg^{2+}. As the CK reaction is probably maintained at near equilibrium,[46-48] the value of the mass action ratio (ϒ) will approximate this constant and, ignoring magnesium (Mg^{2+}), will be given by equation 4.

$$\Upsilon = \frac{[\text{creatine}]\,[\sum \text{ATP}]}{[\text{PCr}]\,[\sum \text{ADP}]\,[\text{H}^+]}$$

If the assumptions listed above are made, this also allows the level of ADP that exists at rest and throughout any period of exercise and recovery to be calculated despite the ^{31}P-NMR spectrum being free from a corresponding peak.

4. Relative Versus Absolute Concentrations of ATP and PCr

Most of the research included in this review has examined changes in [PCr] and [ATP] in relation to those values observed at rest. However, a number of studies have used either ^{31}P-NMR[5,45,49,50] or needle biopsy,[2,51] to establish concentrations of high-energy phosphorylated compounds in absolute terms; these will now be briefly reviewed. The point must be made, however, that a ^{31}P-NMR spectrum gives the concentration of metabolites relative to each other, and therefore the calculation of absolute concentrations in these studies requires the aforementioned assumptions to be made.

One finding that is consistent throughout the literature is that PCr exists in the resting human muscle cell at four to five times the concentration of ATP.[5,45,49] However, as explained above, [ATP] is more resilient to change and is rarely reduced to less than 70% of resting values, even following the most demanding of tasks. In contrast, given that PCr buffers [ATP] by rephosphorylating ADP, it can be substantially depleted during high-intensity exercise. McCann et al.[5] who examined the flexor digitorum superficialis (FDS) in four sedentary adult males (27 ± 3.4 years), reported resting [ATP] and [PCr] of 5.2 and 24.1 mmol/kg wet muscle, respectively. However, after approximately 250 seconds of wrist flexion, these concentrations had fallen to 5.1 and 9.3 mmol/kg wet muscle, respectively. These values correspond to a resting [PCr] of approximately 460% of that of resting [ATP]. Yet on completion of intense exercise, [PCr] had fallen by over 60% while [ATP] had remained essentially unchanged.

Similar differences in resting [PCr] and [ATP] were reported by Taylor et al.[49] who, while also examining FDS, found a resting [PCr] to [ATP] ratio of 4.44 ± 0.43 in a group of sedentary males aged between 22 and 45 years.

Casey et al.[2] used biopsy samples to examine the concentration of phosphorylated metabolites and glycolytic intermediates in the vastus lateralis muscle of nine male volunteers. Muscle was sam-

pled immediately before and after each of two 30-second cycle sprints separated by 4 minutes of passive recovery. Resting values of 83.9 ± 3.6 and 23.9 ± 0.4 mmol/kg of dry matter (mmol/kg D) for [PCr] and [ATP] fell to 34.8 ± 4.5 and 18.7 ± 1.3 mmol/kg D, respectively, following the first 30-second sprint. A further decline to 24.2 ± 1.9 and to 17.3 ± 1.0 mmol/kg D ([PCr] and [ATP], respectively) was reported immediately after the second sprint. Both the resting and post-exercise [PCr] and [ATP] are in close agreement with those obtained by ^{31}P-NMR discussed earlier. Although [ATP] significantly fell during exercise ($p < 0.01$), the decline was relatively small when compared with the drop in [PCr]. While [PCr] fell by 59% and then 71% of resting values, [ATP] fell by only 22 and 28%. Similar changes in [ATP] and [PCr] in response to high-intensity exercise have been reported using biochemical analysis.[4,22]

In addition to the difference between resting [ATP] and [PCr] in muscle cells, there is also a significant difference in the concentrations of these high-energy phosphates between fibre types. In summary of data available in the literature, Fitts[39] reported that in those resting muscle fibres identified as fast twitch (FT), there was a mean of 27 and 90 mmol/kg D for [ATP] and [PCr], respectively, compared with 19 and 58 mmol/kg D, respectively, for those classed as slow. This equates to 42% more ATP and 55% more PCr in type II (FT) fibres, when compared with type I [slow-twitch (ST)] fibres.

5. PCr and the Nature of its Resynthesis

Almost immediately following the onset of muscular contraction, PCr is broken down to maintain [ATP]; the extent of PCr breakdown is primarily dependent on the intensity of muscle contraction. In addition to the energy demand that determines the kinetics of PCr use, a number of other metabolites will effect the CK equilibrium, and indirectly influence PCr recovery.

Di Prampero and Margaria[52] examined O_2 consumption of the isolated gastrocnemius muscle of 30 anaesthetised dogs following tetanic stimulation of the motor nerve that resulted in 0.2 seconds of tetani. It was assumed that the muscle phosphagen store was repaid in accordance with the law of exponential decay and a satisfactory fit of the data was obtained using a single exponential function. Unfortunately, the authors did not report the time-span for which [PCr] was monitored. The importance of this oversight is especially relevant in light of the results obtained by Harris et al.[53] This group reported that PCr resynthesis following both dynamic and isometric exercise was biphasic and therefore better described by an equation of the form given in equation 5, rather than by a mono-exponential function.

$$[PCr]_t = R - D \bullet [c_1 \exp^{-k_1 \bullet t} + c_2 \exp^{-k_2 \bullet t}]$$

where k_1 and k_2 (in minute^{-1}) are rate constants of the two exponential terms and the dimensionless parameters c_1 and c_2 give a sum of one. The time (in minutes) after termination of exercise, is given by t. R is a constant which is the [PCr] of the muscle at infinite time which is deemed to be the same as that which was present at rest prior to exercise. D is also a constant and at the time when exercise is terminated (i.e. t = 0) will predict the absolute decrease in the PCr store below the resting content. When Harris et al.[53] treated their data using this expression, a fast component of PCr recovery with a $t_{½}$ of 21 seconds after dynamic exercise and of 22.5 seconds after isometric exercise and a slow component with a $t_{½}$ in excess of 170 seconds for both was found. It was suggested that if Di Prampero and Margaria,[52] had monitored recovery for a longer duration, a more complex mathematical model would have been needed to describe PCr resynthesis.[53] The authors also suggested that if a mono-exponential model is used to estimate $t_{½}$ of PCr repayment, the $t_{½}$ value will lie somewhere between the two fast and slow extremes. The exact location between the fast and slow $t_{½}$ (i.e. the single $t_{½}$ obtained from the data), will therefore be dependent upon the period of recovery for which data was collected. This dependency was suggested as being responsible for the 'considerable

variation' in values that have previously been reported for the $t_{½}$ of PCr recovery. Possible causes for a biphasic recovery of PCr were briefly discussed by Harris et al.[53] and these are incorporated into the next section of this review.

Taylor et al.,[49] in one of the first studies to use ^{31}P-NMR to examine muscle metabolism, also drew attention to results that indicate an apparent biphasic recovery of PCr. After investigating the kinetics of phosphorus metabolites during recovery from exercise in the FDS, it was found that the obtained $t_{½}$ of PCr recovery was dependent on the method used to treat the data. If analysed as a single set, the data resulted in an increased $t_{½}$ (from 36 to 48 seconds), leaving the investigators to conclude that the extent of resynthesis occurring in the first minute of recovery was being underestimated.

However, Mahler[54] reported findings that were in contrast to those of Taylor et al.[49] and Harris et al.[53] After examining the kinetics of O_2 consumption in relation to the changes in [PCr] in the first 4 minutes following a period of electrically-induced isometric tetanus in frog satorius muscle, it was reported that the mitochondrial CK reaction obeyed a first-order rate law during recovery from contraction.[54] The resynthesis of PCr as described by Di Prampero and Margaria[52] and Mahler[54] can be expressed in the form (equation 6):

$$[PCr]_t = R - D \bullet \exp^{-k \bullet t}$$

where R represents the individual's PCr concentration at rest, R-D indicates the concentration of PCr at time 0, and k is the rate constant. t represents the time after the termination of exercise.

Meyer[55] used ^{31}P-NMR to measure [PCr] in the gastrocnemius muscles of pentobarbital-anaesthetised rats before, during and for 6.5 minutes following 8 minutes of submaximal electronic twitch stimulation at rates of 0.25, 0.5 and 0.75Hz. The mono-exponential recovery curves for the three rates had a single time constant (t_c) of 1.45 ± 0.13, 1.68 ± 0.09 and 1.34 ± 0.05 minutes, respectively. While there was no statistical difference between these constants or with those of PCr depletion calculated at the onset of exercise, the most important point in the context of this review is that recovery of PCr was satisfactorily described by a first-order exponential equation. However, two points appear to be worth noting. First, pH_i [± standard deviation (SD)] varied by only a maximum of –0.16 ± 0.05 units implying that the intensity of muscle contraction was low. In addition, no mention was made as to whether or not a statistical test was administered to determine if a biphasic exponential equation better described recovery of this high-energy phosphate.

However, Nevill et al.[56] did report attempts to fit PCr recovery data from two separate studies with both a mono- and biphasic equation. The first study involved 20 maximal electrical stimulations of the anterior tibialis of three physically active individuals 30 seconds after a pneumatic cuff had been placed around the proximal portion of the thigh and inflated to 250mm Hg. A complete ^{31}P-NMR spectrum was obtained throughout 10 minutes of recovery. The second study involved reanalysis of muscle tissue taken from the vastus lateralis as part of a previously reported study.[22] Muscle was sampled from eight individuals immediately before, immediately after, and then again 30, 180 and 360 seconds following a 30-second maximal sprint on a cycle ergometer. Although a mono-exponential curve described the recovery of PCr following exercise, the addition of a second exponential term significantly improved the quality of fit for data gained in each of the two studies. Although pH_i was not reported in either study, it is nonetheless possible to gain an idea of the extent of intracellular acidosis as an indicator of the intensity of muscle contraction. As stated above, the muscle that was being examined in the second study had been sampled as part of previous work. This previous study[22] had reported that in eight individuals, mean muscle pH_i fell to 6.72 ± 0.06 ($p < 0.01$) following exercise and recovered only slightly, yet nonsignificantly to 6.79 ± 0.02 in the initial 6 minutes of recovery. Given the relatively small SD for both these mean values, it seems rea-

sonable to assume they provide an accurate reflection of the conditions experienced within the three samples re-examined by Nevill et al.[56] In the other component of the study,[56] contraction stimulation was at a frequency of 50Hz and classed as 'maximal exercise' by the authors. These results, therefore, taken along with the studies discussed above, support the proposal that when contraction is severe and there is a significant drop in the pH_i, PCr recovery has both a fast and slow component and therefore is best described by a double exponential equation.

Because of the difficulties associated with 'multiple biopsies' and the noncontinuous nature of serial muscle samples when compared with ^{31}P-NMR, research using biopsy data that has consistently shown the existence of both fast and slow components of PCr recovery, is usually inconclusive. However, examination of the results reported by Bogdanis et al.,[22] who sampled muscle via needle biopsy, seem to support a biphasic recovery of PCr following intense exercise. Eight physically active men (23 ± 2 years) performed a maximal 30-second sprint on a modified friction-braked cycle ergometer against a resistance of 75 g/kg of body mass. Muscle was sampled from the vastus lateralis muscle before, immediately after and then again 90, 180 and 360 seconds following exercise. PCr was reduced to 19.7 ± 1.2% of pre-exercise levels at the end of the maximal sprint, then increased rapidly to 65.0 ± 2.8% of resting levels after only 90 seconds of recovery. However, after a further 4.5 minutes of recovery, the final biopsy indicated that [PCr] was still only 85.5 ± 3.5% of pre-exercise levels; indeed the mathematical models derived from the data predicted that PCr resynthesis in the participants would not be 95% complete until 13.6 minutes of recovery had elapsed. It is interesting to note that the $t_{1/2}$ of PCr resynthesis for the slow component as reported by Harris et al.[53] was more that 170 seconds and mention was made earlier that any $t_{1/2}$ calculated with a single exponential function would arrive at a value somewhere between that of the fast and slow component, depending on the time duration for which [PCr] was monitored. As Bogdanis et al.[22] derived the $t_{1/2}$ of 56 seconds from an equation with a single exponential term over 6 minutes of recovery, it appears highly likely that this value was somewhere between the $t_{1/2}$ for the fast and slow components that would have been obtained had a biphasic model been used.

This section to date can be summarised as follows: whenever contraction is intense and when pH_i and [ATP] significantly change, there appears to be an initial fast phase of PCr resynthesis followed by a second slower phase of recovery. Furthermore, the recovery of PCr appears to be best described by a double exponential equation similar to that shown in equation 5. In contrast, a first-order exponential equation (equation 6) appears to best describe the kinetics of PCr recovery following low-intensity contraction where pH_i does not significantly fall.

Although this may appear to neatly explain the nature of PCr resynthesis and suggest that it is either mono- or biphasic depending on the exercise and nature of the preceding exercise, data reported by Newcomer et al.[57] appear to suggest that the solution is not so simple. As technology has improved, the ability of ^{31}P-NMR units to utilise smaller and smaller time resolutions has become more advanced. By using a time resolution of 0.5 seconds, Newcomer et al.[57] showed that even using a slope calculated over the initial 10 seconds of recovery, initial PCr resynthesis rate is underestimated by as much as 56%. In fact, the slope describing PCr resynthesis during the first 3 seconds of recovery was statistically different ($p = 0.001$) from the slope that was calculated during the first 0.5 seconds. This suggests that under some circumstances, even a double exponential model may not adequately describe the resynthesis process.

6. The Effect of pH_i on PCr Recovery

Since H^+ is released in the resynthesis of PCr (equation 2), it is reasonable to expect pH_i to influence the position of the CK equilibrium because of

the resultant presence of H^+ in the mass action ratio (equation 4).

Harris et al.[53] calculated the initial velocity of PCr resynthesis after exercise as 2 to 3 mmol/kg D/sec *in vivo*, which was only a fraction of the theoretical rate of the maximum velocity of CK calculated from *in vitro* studies. It was therefore proposed that factors other than enzymatic activity must regulate PCr resynthesis during recovery from high-intensity exercise. Citing then recent studies that had suggested a similar recovery time between PCr and muscle pH of around 20 minutes following maximal dynamic exercise, Harris et al.[53] proposed that the limiting factor may be the effect of pH_i on the CK equilibrium. However, it was also conceded that as there were differences in the form of these two recovery time courses, other possibilities did exist.

Around the same time, Sahlin et al.[58] reported data collected from seven men aged between 20 and 27 years. Two of the three exercise protocols used in the study involved a combination of occluded circulation and isometric contraction of the knee extensors at 68.3 ± 1.3% of maximum voluntary contraction (MVC) for 25 seconds or until volitional fatigue. The remaining protocol induced changes in the [PCr] and pH_i by using 15 minutes of occluded circulation only. Muscle samples were taken in all three experiments at various times, which were unique to the different protocols. The major finding of the study was that, irrespective of the protocol used to induce metabolic change in the muscle, there was a significant correlation of $r = 0.92$ ($p < 0.01$; $n = 34$) between muscle pH_i and the CK equilibrium. This led the authors to conclude that a change in pH_i is responsible for the altered state of the CK reaction and is therefore largely responsible for determining [PCr].

In a similar study, Sahlin et al.[59] used a different exercise protocol, but again concluded that pH_i affects the CK equilibrium and thus the recovery of PCr. Nine men (18 to 27 years) performed exhaustive exercise on an electrically-braked cycle ergometer following one of two protocols. These two protocols required the participants to pedal to exhaustion at workloads of either 305W (protocol one) or 300W (protocol two); average time to exhaustion was 6.5 and 7.7 minutes for the two exercise bouts respectively. Muscle was sampled from the quadriceps femoris at 1, 3, 7 and 14 minutes following the end of exercise in protocol one, and immediately post-exercise and then again a further two or three times during the subsequent 10-minute recovery period during protocol two. Normal circulation was occluded for 1 minute immediately before each muscle biopsy in protocol one but not at all occluded during protocol two. Consequently, in the first procedure, muscle was sampled after having 0.1, 1, 4 and 10 minutes of intact circulation. It was found that, provided data from the muscle which was sampled at 0.1 minutes were ignored, there was a significant relationship ($r = 0.85$; $p < 0.01$; $n = 24$) between muscle pH and log ([creatine][ATP]/[creatine phosphate][ADP]). This led the authors to conclude that the latter stages of PCr recovery were limited by the recovery of muscle pH.[59] A secondary component of the study, discussed in the next section, yielded evidence suggesting that it is O_2 availability that limits the initial phase of PCr recovery following exercise.

Taylor et al.,[45,49] and Arnold et al.[50] were among the first researchers to examine the relationship between PCr resynthesis and pH_i using ^{31}P-NMR. As PCr has an ionisation constant (pK_a) far removed from physiological pH_i, the position of its peak on the ^{31}P-NMR spectrum remains constant throughout exercise and recovery. However, as the pK_a for P_i approximates pH_i, the position of the ^{31}P-NMR peak for P_i is pH_i-dependant. The difference between the two peaks, in parts per million, is referred to as the chemical shift and can be used to determine pH_i using equation 7:

$$pH_i = 6.75 + \frac{\log(\sigma - 3.27)}{(5.69 - \sigma)}$$

where σ represents the chemical shift of P_i relative to PCr.

Taylor et al.,[49] after examining the concentrations of metabolites in the FDS of sedentary adults,

reported that the $t_{1/2}$ of PCr resynthesis was greatly increased following exercise that resulted in a very low pH_i. The authors did not discuss possible reasons for this phenomena.

Arnold et al.[50] examined the kinetics of PCr recovery following two exercise protocols that were similar in all respects except for exercise intensity. Participants squeezed the rubber bulb of a sphygmomanometer until empty, then immediately released it. This was continued for approximately 270 seconds at 100mm Hg (light exercise) and 150 seconds at 500mm Hg (heavy exercise). Data collected showed that [PCr] ± SD was depleted to 55.8 ± 8 and 33 ± 4% of resting concentration for these two exercise intensities, respectively. The ^{31}P-NMR spectrum, which was reflective of FDS, showed that in both cases pH_i rose initially following the onset of exercise; a common occurrence attributed to the absorption of H^+ by PCr breakdown (equation 2). At the end of exercise, the ^{31}P-NMR spectrum revealed that following the light and heavy protocols respectively, pH_i had fallen to values of 6.88 ± 0.02 and 6.23 ± 0.08, respectively. Because [PCr] was reduced to a lower level following heavy exercise, the quantity of PCr that was resynthesised during recovery from this bout was greater. However, when the rate of PCr resynthesis (d[PCr]/dt) was compared, it was slower following the heavy protocol (i.e. the trial in which pH_i fell to a lower level). In initial discussions of the then recent literature, it was proposed that either pH_i or [ADP] were responsible for any observed effect of exercise intensity on PCr resynthesis. However, while [ADP] increased during both regimens, the $t_{1/2}$ of its recovery was not significantly different between the two trials. In addition, it was noted that ADP recovery was faster than that of PCr and its concentration was not significantly different from resting levels after only 2 minutes of recovery. This led the authors to conclude that the difference in PCr recovery rates is determined primarily by the extent of intracellular acidosis.

Bendahan et al.[60] also examined metabolic changes associated with exercise and subsequent recovery in FDS using ^{31}P-NMR. Fourteen volunteers performed finger flexion at 1.5-second intervals for 3 minutes lifting a weight of 6kg with their dominant arm. This protocol resulted in differing metabolic responses amongst participants and, as a consequence, three distinct groups were formed on the basis of end-exercise pH_i. Mean pH_i ± SD for the three groups were 6.78 ± 0.05, 6.62 ± 0.04 and 6.33 ± 0.07, respectively. Correlations of r = 0.90 and r = 0.85 (n = 14) between end-exercise pH_i and both the $t_{1/2}$ of PCr recovery and average rate (d[PCr]/dt) respectively were reported. This led Bendahan et al.[60] to conclude that the extent of intracellular acidosis reached at the end of exercise determined the rate of PCr resynthesis.

Takahashi et al.[6] used ^{31}P-NMR to examine the relationship between exercise intensity and PCr resynthesis in the quadriceps muscle group in five distance runners (23.2 ± 0.4 years) and seven non-endurance-trained controls (25.4 ± 0.8 years) following an incremental bout of knee extension. PCr was reduced during four exercise bouts to levels that were determined by comparing the height of the increasing and decreasing P_i and PCr peaks respectively on the ^{31}P-NMR spectrum. Exercise was terminated when the P_i peak was: (i) half the height of the PCr peak (light exercise); (ii) slightly lower than the height of the PCr peak (moderate exercise); and (iii) higher than the PCr peak (severe exercise). A fourth exercise bout was also completed which was terminated at volitional fatigue and classed as exhaustive. pH_i continued to fall during the early stages of recovery and values at the end of exercise therefore were found to be higher than the minimal pH_i that resulted some time later. The mean time between the end of exercise and when pH_i reached its lowest value was dependent on both the exercise intensity and the training status of the study group; these time periods varied from 45.2 ± 9.7 seconds following light exercise to 116.1 ± 8.6 seconds following exhaustive exercise. As glycolysis ceases when contraction is terminated,[49,53,61] the continued fall in pH_i is attributed to the release of protons during the

resynthesis of PCr (equation 2). Low (–0.02 and –0.46) correlations ($p > 0.05$) between the t_c of PCr recovery and minimal pH_i were reported by Takahashi et al.[6] for light and moderate exercise, respectively. However, following the two exercise trials classed as severe and exhaustive, significant correlations of $r = –0.71$ ($p < 0.05$) and $r = –0.83$ ($p < 0.01$), respectively were reported. These findings suggest that, at least during part of the resynthesis process, the rate of PCr resynthesis is being impaired by intracellular acidosis.

McCann et al.[5] studied the system linearity of the CK reaction in the right forearm (FDS) of four adult men (27 ± 3.4 years) in an attempt to determine if the t_c describing PCr and P_i kinetics were independent of work rate. ^{31}P-NMR techniques were used while individuals performed wrist flexion and extension on a specifically designed ergometer. Exercise consisted of five identical bouts each lasting approximately 250 seconds followed by 10 to 15 minutes recovery. Using computerised digital analysis on the force and distance trace for a contraction cycle, a mean work rate of 1.7 ± 0.13 and 3.6 ± 0.24W for exercise protocols classed as moderate and heavy, respectively, were calculated. The t_c for both [PCr] and [P_i] was calculated during, and then again following 'moderate-intensity' wrist flexion in an attempt to predict the changes in these metabolite concentrations during and following a subsequent bout of 'heavy' exercise. The underestimation of PCr breakdown during the work phase of heavy exercise was associated with an increased [H^+] as predicted by the CK equilibrium reaction. Given this, it appears reasonable to predict that the elevated [H^+] during exercise would then inhibit PCr resynthesis during recovery. The observations that the net recovery of [PCr] following heavy exercise was greatly overestimated by the linear model t_c (derived from moderate intensity exercise), and that 15 minutes were required to allow complete recovery of PCr are consistent with this position. Nevertheless, the effect of a decreased pH_i was difficult to quantify as the P_i resonance peak would often 'undershoot' the initial resting levels and therefore recede into the noise of the spectrum, making pHi difficult to determine.

While suggesting that pH_i probably regulates the recovery of the CK equilibrium, a number of studies reviewed previously[45,50] have also observed a depletion in [ATP] and [ADP] in response to exercise; this makes the exact mechanisms responsible for limiting the rate of PCr resynthesis difficult to determine. However, in the study by McCann et al.,[5] [ATP] was not significantly depleted, and was not therefore considered as a possible influence on the recovery of PCr. In addition, [ADP] had completely recovered 180 seconds into recovery and as the slow recovery phase of PCr continued for well in excess of this period, the concentration of this metabolite was also ruled out as an influencing factor. These results were among the first to show that PCr resynthesis is inhibited by intracellular acidosis during recovery.

Consistent with the view that pH inhibits the latter stages of PCr recovery, Lodi et al.[62] report a strong relationship ($r = 0.89$, $p < 0.01$) between the minimum pH of the muscle, reached sometime during the early phase of recovery, and the t_c of PCr resynthesis calculated during the first 5 minutes following the termination of exercise.

The review to date has examined some of the early literature responsible for the current view that following intense exercise that causes a fall in pH_i, a fast and slow component of PCr recovery exists and that consequently, a mono-exponential equation will at first underestimate and then over estimate the extent of PCr resynthesis. The literature does not appear particularly divided on the role of acidosis on PCr recovery, with most research data indicating that an elevated [H^+] is associated with a reduced rate of PCr resynthesis. Consequently, PCr recovery will be satisfactorily described by a mono-exponential equation providing pH_i has not been significantly reduced. However, given that most of the studies reviewed have reported rapid recovery of [PCr] while pH_i is either at its lowest point, or is still falling,[6,24,25] some

other factor must control the kinetics of PCr resynthesis in the initial stages of recovery following intense exercise.

Walter et al.[9] examined the relationship between the rate of PCr resynthesis following intense exercise and the oxidative capacity of skeletal muscle; three different exercise protocols were used to significantly reduce [PCr]. The three protocols had differing effects on pH_i and it was reported that under conditions in which the pH_i was decreased, PCr recovery kinetics became increasingly biphasic. For example, following the most intense protocol, pH_i was lowered to 6.45 ± 0.07; the line describing recovery of PCr in the resultant 10 minutes was a significantly better fit ($p < 0.05$) when using a bi-exponential compared with a mono-exponential function. The rate constant of the PCr recovery curve (k) over the 10-minute recovery period following the different protocols was then used to estimate muscle oxidative capacity (V_{max}) within the medial gastrocnemius muscle of the eight participants (19 to 27 years). The results showed that where recovery became biphasic because of intracellular acidosis, any model that uses k to predict V_{max} yields an underestimation of this value. These results are consistent with the earlier suggestion that a high $[H^+]$ inhibits the resynthesis process.

However, despite an end-exercise difference of 0.59 pH units between two of the exercise protocols,[9] the measured initial PCr resynthesis rates remained similar, suggesting that this initial phase was unaffected by intracellular acidosis. A change in pH_i therefore separates the direct relationship between k and V_{max} in all but the initial stages of PCr recovery. Along with the studies reviewed above, this suggests that the pH-induced inhibition on the recovery of the CK equilibrium is influential only during the later, slower component of recovery. Roussell et al.[63] reported data that support this conclusion. Thirteen individuals performed a bout of low-frequency (LF, moderate exercise) and high-frequency (HF, heavy exercise) muscular contraction which enabled the rate constant (k_{PCr}) of PCr recovery to be examined using ^{31}P-NMR during 20 minutes of passive recovery. It was reported that there was a large variation between individuals (variation coefficients: 43 *vs* 57% for LF and HF, respectively) and between protocols ($k_{PCr} = 1.3 \pm 0.5$ *vs* 0.9 ± 0.5 min^{-1} for LF and HF, respectively) and that the large interindividual variability could be described by a linear relationship between k_{PCr}, the amount of PCr consumed during exercise and the end-exercise pH_i ($p = 0.0007$). As k_{PCr} was derived over a 20-minute recovery period using a single exponential curve, this result further supports the conclusion that pH_i plays a significant role in determining the rate of PCr recovery during recovery from high-intensity exercise. However, as was reported by Walter et al.,[9] the initial rate of PCr recovery was always constant irrespective of the metabolic conditions within the muscle cell at the end of exercise.[63]

The possibility exists that this well accepted slower resynthesis phase of PCr during the secondary stage of recovery may have been caused by a loss from the adenine nucleotide pool during exercise; a reduced [ATP] would manifest itself in a decreased ability to rephosphorylate creatine. However, a biphasic recovery of PCr has still been observed when [ATP] remains at resting levels,[5,45] suggesting that although any decline in [ATP] would influence the absolute rate of both phases, the slow component is inhibited by acidosis while the initial kinetics of PCr recovery is regulated by some other factor.

7. The Importance of O_2 for PCr Resynthesis

The importance of O_2 for PCr resynthesis was first established by Harris et al.[53] who used a pneumatic cuff placed around the thigh (inflated to 240mm Hg); PCr resynthesis was examined following a bout of isometric knee extension. Muscle was sampled on three occasions during 6 minutes of ischaemic recovery. The data showed that PCr resynthesis had been completely suppressed. In a parallel study from which results were also re-

ported, the pneumatic cuff was deflated for 25 seconds after 90 seconds of occluded recovery and then re-inflated. Under these conditions, PCr had recovered to levels that could have been expected following 25 seconds of recovery with normal circulation.

In addition to their own observations, the results from two other studies were also discussed. The $t_{1/2}$ of O_2 debt repayment in humans had been previously established to be around 25 seconds by Margaria et al.[64] and the observation was made that this was close to the $t_{1/2}$ of PCr recovery observed during their own work.[53] In addition, the point was made that there was a quantitative agreement between excess O_2 consumption during recovery and PCr restoration in dog muscle.[65] When results from these other studies were considered with their own data, Harris et al.[53] concluded that the ATP used in the resynthesis of PCr is derived from oxidative metabolism.

Sahlin et al.[59] examined the relationship between O_2 availability and PCr resynthesis. Three muscle samples were obtained within 1 minute of an exercise protocol that required participants (n = 4) to isometrically contract the knee extensors to fatigue (62% MVC). During contraction, and the first minute of recovery while muscle was being sampled, local blood flow to the leg was occluded by means of a cuff placed around the upper thigh and inflated to 260mm Hg. One muscle sample was immediately frozen and the other two incubated in either an oxygen rich (95% O_2 + 5% N_2) or an oxygen deprived (5% CO_2 + 95% N_2) environment for 15 minutes. It was found that [PCr] had increased from 4 to 68% of the mean resting value during the incubation period in the oxygen rich environment.[59] In addition, the CK mass action ratio had decreased to a level equivalent to that reflective of 1 minute of recovery with intact circulation. In contrast, PCr recovery was not observed in the samples incubated in an oxygen deprived environment (95% N_2 + 5% CO_2). When these data were considered with the data previously reviewed, the authors concluded that the fast component of PCr recovery is limited by O_2 availability.

As previously reviewed in section 6, Taylor et al.[49] utilised ^{31}P-NMR to examine the kinetics of PCr recovery within the FDS of five participants who were required to squeeze a rubber bulb for 1 minute at an intensity classed as light by the authors. Both the 1 minute of exercise and the following 6 minutes of recovery were performed under ischaemic conditions. The fact that PCr resynthesis was completely suppressed during the ischaemic recovery period led the authors to conclude that, at the termination of exercise, glycolysis immediately ceases, despite increases in the concentration of AMP and ADP. Although the likely reasons for the termination of glycolysis under these conditions were discussed, only one conclusion was drawn regarding the lack of PCr resynthesis. The authors concluded that the recovery of PCr following nonfatiguing exercise is almost exclusively oxygen-dependent and that as a result, the time-course of metabolic recovery following exercise provides information about the oxidative capacity of skeletal muscle.

Quistorff et al.[61] also used a cuff to prevent normal blood flow and to examine PCr resynthesis. Eight male participants performed a single isometric contraction for 60 or 30 seconds, at an intensity that was either 70 or 90% of MVC, respectively. This caused a fall in [PCr] to between 40 to 60% of resting levels for all individuals. The cuff, which was placed around the top of the thigh and inflated to a pressure of 32 kPa, ensured ischaemic conditions for both contraction and the initial period of recovery that approximated 5 minutes. This was then followed by the removal of the cuff and a further 2 to 5 minutes of recovery under conditions of normal blood flow. The principal finding was that glycolysis ceased the moment exercise was terminated, which was consistent with the results from Harris et al.[53] and Taylor et al.[49] and supported the proposal that the ATP used to rephosphorylate creatine is derived from aerobic metabolism only.

Apart from studies that have involved prevention of normal blood flow, resulting in impaired oxygen delivery, there is a plethora of experimental findings throughout the literature which lend weight to the importance of O_2 delivery in the resynthesis of PCr. Tesch et al.[3] examined the [PCr] in pools of freeze-dried FT and ST fibres obtained by muscle biopsies from the vastus lateralis muscle of 12 physically active men. Muscle was sampled at rest, immediately after exercise (which consisted of 30 maximal voluntary knee extensions at constant angular velocity 3.14 rads/sec), and then again after 60 seconds of passive recovery. There was no difference in PCr levels between fast (often referred to in the literature as FG, white or type IIb) and ST fibres (SO, red or type I) immediately following exercise (25.4 ± 19.8 *vs* 29.7 ± 14.4 mmol/kg D, respectively). However, after 60 seconds of recovery, the ST fibres had a significantly greater level ($p < 0.05$) of PCr relative to the initial level when compared with the FT fibres; [PCr] ± SD had recovered to 92 ± 4.6% mmol/kg D of resting concentration in the ST fibres yet was only 66.3 ± 7.6% mmol/kg D in the FT fibres. The authors concluded that the faster rate of PCr resynthesis in the ST fibres was due to their higher potential for oxidative phosphorylation.[66,67]

The data reported by Jansson et al.[1] are consistent with this conclusion; these authors examined the relationship between the oxidative potential of the vastus lateralis muscle and the recovery of muscle metabolites following intense exercise.[1] Eleven male volunteers performed three bouts of 30 maximal unilateral knee extensions; muscle was sampled before bout one, immediately after bout two and then again immediately before bout three. Exercise was performed at 3.14 rad/sec on a Cybex™ isokinetic dynamometer and each bout was separated from the next by 60 seconds of passive recovery. A relationship ($r = 0.79$, $p < 0.05$) was found between citrate synthase (CS) activity and the recovery of PCr following bout two.

While these results support a causal relationship between the rate of PCr resynthesis and the potential for oxidative phosphorylation, they should be interpreted with caution; they are in direct contrast with those reported by Dawson et al.,[68] who examined the rate of PCr repletion following one and five 6-second bouts of maximal sprinting on a cycle ergometer. Despite PCr levels (±SD) falling to 44.9 ± 6.4 and 21.1 ± 5.6 mmol/kg D 10 seconds following these two bouts respectively, there was no relationship between the rate of its repletion during the subsequent 3 minutes of recovery and the activity of CS.

Casey et al.[2] also sampled muscle at rest, and then again following a maximal bout of exercise. Nine male cyclists performed two 30-second cycle sprints each separated by 4 minutes of passive recovery. The data, which support the findings of Tesch et al.,[3] show that relative to end-exercise values, resynthesis of PCr was faster in type I muscle fibres.[2] It seems probable that the increased O_2 availability and potential for oxidative metabolism in the type I fibres, due to factors such as a greater capillarisation,[69,70] and a higher mitochondrial density[66] with associated oxidative enzyme activity,[67] were responsible for the observed differences. However, Casey et al.[2] acknowledged that the exact mechanism responsible was not known and suggested that the slower rate of resynthesis may also have been caused by the greater depletion of ATP within the type II fibres in the earlier exercise bout. This is possible given that there was a significant relationship ($r = 0.89$, $p < 0.01$) between the recovery of ATP and the resynthesis of PCr in both fibre types.

The material reviewed to this point overwhelmingly supports the importance of O_2 in the resynthesis of PCr; glycolysis ceases at the termination of exercise, and oxidative phosphorylation provides the ATP required for PCr resynthesis. If, as the literature suggests, the capacity for oxidative metabolism is the dominant influence on the rate of PCr resynthesis in the early part of recovery following exercise, it follows that any change in this capacity should manifest itself in a corresponding change in the ability to resynthesise PCr.

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system. It has been shown that during electrically stimulated exercise, there is excessive fatigue and greater PCr depletion in individuals with MS when compared with controls.[71,72] This phenomena is thought to result from secondary changes within the muscle caused by de-conditioning from disuse. If this is the case, and oxidative metabolism does indeed determine the rate of PCr resynthesis in the initial stages of recovery, it appears reasonable to suggest that MS patients who have poorly developed potential for oxidative metabolism would, as a result of their condition, have slower kinetics of PCr recovery following intense exercise. Kent-Braun et al.[73] tested this hypothesis using 13 individuals with MS and 8 healthy controls. Because of the inability of the MS patients to perform incremental exercise, PCr was depleted by electrical stimulation (surface electrode) of the dorsiflexors at the peroneal nerve. In the control group, however, as a protocol of this nature had previously proven ineffective in depleting PCr, participants performed intermittent isometric contractions of the dorsiflexors. There was no significant difference in the ability of the different protocols to deplete PCr, as reflected by post-exercise [PCr] of 49.7 ± 4.8 versus 45.5 ± 5.3% resting levels ($p > 0.05$) for the control group and MS patients, respectively. In addition, the difference in post-exercise pH_i was only 0.02 pH units ($p > 0.05$). The $t_{1/2}$ of PCr resynthesis in the 10 minutes for which recovery was monitored (via ^{31}P-NMR), was significantly greater ($p < 0.02$) for the MS patients when compared with the controls. As pH_i did not significantly change as a result of exercise and electrical stimulation, the review to this point, and in particular the results from Mahler[54] and Meyer[55] indicate that the use of a $t_{1/2}$ of PCr resynthesis is justified, as recovery was most likely mono-exponential in nature. The results from Kent-Braun et al.,[73] therefore, providing that individuals with MS do have deconditioned peripheral oxidative capacity, provide further evidence that support a close relationship between the peripheral component of aerobic metabolism and PCr resynthesis during the initial period of recovery following intense exercise.

If the above evidence suggesting that the limitation to the resynthesis of PCr may be peripherally located is accepted, then results reported by McCulley et al.[42] seem to provide additional indication as to its possible location. These researchers used both ^{31}P-NMR and near red spectroscopy (NRS) to examine the relationship between muscle metabolism and muscle oxygen saturation. NRS is a technique that monitors oxygen saturation of haemoglobin and myoglobin in skeletal muscle using the fact that light absorption by the haem groups of these compounds varies with the amount of oxygen that is bound to them. Results showed that the resynthesis of PCr was slower than the recovery of oxygen saturation within the wrist and plantar flexors of the four individuals involved (22 to 44 years). This suggests that if the limitation for PCr resynthesis is a peripherally located component of oxidative phosphorylation, then it is one that is involved with the use of oxygen as an electron acceptor within the muscle cell, rather than with the transport of oxygen into the cell itself. It is worth noting, however, that end-exercise PCr and pH_i levels remained around 50 to 60% of resting levels and 7.0 to 7.1, respectively, which suggests that contraction was not intense.

8. The Role of ADP

Despite the concentration of free ADP being at an insufficient level to register a ^{31}P-NMR signal above the surrounding noise, the kinetics of its changes within the cytosol during both exercise and recovery can still be estimated.

Takahashi et al.[6] used ^{31}P-NMR and a weight and pulley system to monitor the concentration of various muscle metabolites following a graded bout of exhaustive knee extension exercise. Participants maintained a rate of two contractions every 3 seconds against an initial load of 4kg for a period of 3 minutes. Following this, resistance was increased each minute until [PCr] reached one of

four pre-designated levels. Exercise was terminated when the P_i peak was: (i) half the height of the PCr peak (light exercise); (ii) slightly lower than the PCr peak (moderate exercise); and (iii) higher than the PCr peak (severe exercise). A fourth level also existed which was termed exhaustive and which was terminated by the individual when they could no longer continue. The calculated concentration of ADP [± standard error of the mean (SEM)] significantly increased ($p < 0.05$) from 6.4 (± 0.4) μmol/L at rest to 104.7 (± 16.3) μmol/L following one exercise bout in a group of male controls (n = 7) compared with a significant increase ($p < 0.05$) from 7.2 (± 0.5) μmol/L to 68.9 (± 12.0) μmol/L in a group of endurance-trained runners (n = 5). Evidence of the demanding nature of the exercise task is provided by the fact that not only did the [ATP] and pH_i significantly fall ($p < 0.05$) but the PCr : (PCr + P_i) ratio fell to 18.19% of the resting level in the controls ($p < 0.05$). Takahashi et al.[6] did not report the kinetics of ADP recovery. However, examination of the data presented by Wackerhage et al.[74] provides some indication as to the rapid recovery of this compound. After an exercise protocol that also increased the [ADP] to in excess of 100 μmol/L, pre-exercise values of [ADP] were restored within around 60 seconds following the termination of exercise in a group of 11 endurance-trained sports students.[74]

Because of its position in the CK equilibrium, restoration of ADP will be closely related to the resynthesis of PCr. The focus of this review will now move to examining the possible role of [ADP] in regulating the rate of PCr resynthesis. Oxidative phosphorylation within the mitochondria appears to be related to the rate at which PCr is resynthesised during the initial stages of recovery following intense exercise. However, it has been proposed[75-77] that mitochondrial ATP synthesis is controlled by cytosolic free [ADP] and there is a large body of evidence supporting this.[75,78-80] Brindle et al.[75] measured ATP turnover in the rat hindlimb during steady-state isometric contraction using the technique of ^{31}P-NMR magnetisation transfer. After animals were anaesthetised, the sciatic nerve was maximally stimulated for variable periods between 10 to 90msec at a frequency of 0.5, 1 or 2Hz. Metabolite concentrations and pH_i were then measured by ^{31}P-NMR using a 4.3 T vertical-bore magnet. As the period of contraction increased, so too did the [P_i] and [ADP], while [PCr], pH_i, and to a lesser extent [ATP], decreased (significance not reported). The flux between P_i and ATP showed a linear dependence on the calculated free [ADP] up to 90μmol. While the relationship may have continued above this concentration, the dependence of the flux on [ADP] could not be determined at higher concentrations since the hind limb muscle fatigued too rapidly when the tetanic pulse duration exceeded 90msec. This linear dependence on the free [ADP] is consistent with the proposal that mitochondrial ATP synthesis is controlled by [ADP].

Kemp et al.[76] analysed a large number of published studies, including observations from their own laboratory, in an attempt to determine the controlling mechanisms of mitochondrial respiration in skeletal muscle. After ruling out regulation by creatine concentration (as postulated in the PCr shuttle hypothesis), it was noted that PCr resynthesis, in those studies that were examined, had an approximate hyperbolic dependence on [ADP]. As this relationship, with a maximal value of 40 mmol/L cell water and a K_m of around 30 μmol/L cell water, is held throughout recovery, it was proposed that mitochondrial respiration is under kinetic control of adenine nucleotide translocase. Adenine nucleotide translocase is a nuclear encoded protein located in the inner mitochondrial membrane; in the mitochondria of tissues with high energy demands, it can comprise up to 10% of the total inner membrane proteins. Since this carrier catalyses the transmembrane exchange between ATP generated inside mitochondria by oxidative phosphorylation and the cytosolic ADP, it is a key link between the mitochondrial and cytosolic compartments. As this ATP will then be used to rephosphorylate creatine according to equation 2, the authors proposed that

the initial rate of PCr resynthesis at the completion of exercise is controlled by the cytosolic [ADP].[76]

However, Kemp et al.[76] make note of the fact that the initial rate of PCr resynthesis during recovery was also closely related to the free energy of ATP hydrolysis (ΔG_{ATP}), making this another possible regulating influence. The authors consequently conceded that while [ADP] was believed to be the primary regulator of mitochondrial respiration, system constraints make it difficult to separate the relative importance of the two. Indeed the existence of pH in the same equilibrium expression as PCr and ADP, makes it difficult to separate the effects of the different metabolites on mitochondrial respiration.

After acknowledging that for this particular reason, the factors influencing PCr resynthesis are difficult to consider independently of each other, Barstow et al.[81] attempted to investigate the relationship between [PCr], [ADP] and ΔG_{ATP} when changes in $[H^+]$, and therefore pH_i are accounted for. Six individuals performed incremental plantar flexion exercise on a treadle ergometer while ^{31}P-NMR was used to obtain metabolite concentrations and to allow the calculation of ΔG_{ATP} using equation 8:

$$\Delta G_{ATP} = \Delta G_O + RT \ln \frac{[ADP][P_i]}{[ATP]} + RT \ln 10^{pH_{obs} - 7}$$

where ΔG_0 (Gibbs free energy), is –31.8 kJ/mol, R is the gas constant (8.314 J/mol/K) and T = 310°K. Once again, the authors reported results that led to the elimination of the PCr shuttle hypothesis as a regulating mechanism. Reporting data that were similar to those reported by Kemp et al.,[76] the relative importance of cytosolic [ADP] and of ΔG_{ATP} in controlling mitochondrial respiration was again initially difficult to separate. However, it was reported that the relationship between metabolic rate and free cytosolic [ADP] showed a deviation from the expected hyperbolic relationship at around 70% of maximal metabolic rate. This, coupled with the fact that metabolic rate was significantly related to ΔG_{ATP} ($r = 0.997$; $p < 0.01$) throughout its entire range, led the authors to conclude that at higher work rates at least, mitochondrial respiration is being controlled by the cytosolic free energy of ATP hydrolysis; ΔG_{ATP} will decrease with an elevated [ADP].

The results from Krause and Wegener,[82] however, seem to summarise the importance of [ADP] in controlling the PCr resynthesis process. While examining metabolism in frog muscle, it was reported[82] that 30 seconds of swimming was sufficient to increase [ADP] from 14.0×10^{-6} mmol to 156.4×10^{-6} mmol. Despite the integrity of [ATP] being maintained through exercise, ΔG_{ATP} was reduced from –60.61 to –47.90 kJ/mol. This suggests that irrespective of which of the two aforementioned factors (free cytosolic [ADP] or ΔG_{ATP}) are primarily responsible for controlling PCr resynthesis, it seems that [ADP] will be particularly important. It will either act directly through its free cytosolic concentration or indirectly through its effect on the free energy of ATP hydrolysis.

The review to this point suggests that following intense exercise: (i) PCr is resynthesised by ATP produced by oxidative phosphorylation only; (ii) the initial rate of PCr recovery is controlled by the rate of mitochondrial ATP synthesis and is free from inhibition by H^+; (iii) the latter stages of PCr recovery can be inhibited by an increased $[H^+]$, and thus a lowered pH_i, acting through a product inhibition of the reaction facilitated by mitochondrial CK (equation 2); and (iv) PCr recovery is best described by a mono-exponential function when pH_i is not significantly reduced, yet recovers in a more complex fashion when contraction is intense and pH_i remains significantly lowered by the time [ADP] has returned to near resting levels. This initial fast phase is then followed by a slower phase that can have a $t_{½}$ of up to 170 seconds.[59] In addition, it appears that while this initial fast phase is controlled by the rate of oxidative ATP synthesis, the slower phase proceeds at a rate that reflects the much slower rate of pH_i recovery as the concentration of the equilibrium metabolites continually readjust to maintain the mass action ratio at approximately 1.66×10^9 mol^{-1}.

9. Relationship Between PCr Resynthesis and Maximal Aerobic Power

9.1 Results from Previous Work

Endurance-trained individuals are characterised by higher than average values of maximal aerobic power (MAP) as indicated by their elevated maximal oxygen uptake ($\dot{V}O_{2max}$) scores; they are more effective at transporting O_2 to the muscle and in facilitating its movement to the mitochondria where it is used as an electron acceptor during ATP production. Irrespective of whether the driving force for mitochondrial oxidation is the cytosolic free [ADP] or regulation by the free energy of ATP hydrolysis, the review to this point suggests that individuals with an elevated $\dot{V}O_{2max}$ should be better able to resynthesise PCr following a bout of intense exercise. As a result, it is not surprising that the rate of PCr resynthesis following moderate exercise represents the muscle oxidative capacity,[54,55] and has been found to be faster in endurance-trained athletes when compared with sedentary controls.[41,83] Corroborating these results are those of Paggani et al.[84] which showed a significant relationship ($r = 0.84$, $p < 0.01$) between CS activity and the time-constant of PCr recovery in a group of endurance-trained rats. This followed moderate intensity stimulation of the gastrocnemius muscle that did not significantly reduce pH_i. In addition, an 8- to 10-week training period significantly increased both the activity of CS and the rate of PCr resynthesis. Of further support was the fact that a parallel programme involving chemical thyroidectomy, which reduced the activity of this same enzyme by 71%, resulted in a significant reduction in the ability to resynthesise PCr following a bout of the same moderate intensity.

As a result of studies that support a relationship between oxygen availability and PCr resynthesis following low-intensity exercise, the rate of PCr recovery is becoming increasingly important as a diagnostic tool for muscular disorders such as severe mitochondrial myopathy.[50,85] However, when exercise is more intense and there is a fall in pH_i, conflicting results have been reported.[6,25,86] As might be expected from equation 2, an increased $[H^+]$ inhibits the resynthesis of PCr. However, as this inhibition appears to be confined to the later stages of recovery, a higher initial rate of PCr resynthesis following exercise that is intense and causes a significant decline in pH_i rate should still be evident in those with an elevated MAP.

Yoshida and Watari[86] examined PCr resynthesis during recovery from exercise in the biceps flexor of five long-distance runners and six controls. Four 2-minute bouts of biceps flexion at an intensity of 20 kg/m/min were performed, with each bout separated by 2 minutes of passive recovery. There was a significantly smaller ($p < 0.05$) t_c of PCr recovery in the long-distance runners when compared with the controls. Although $\dot{V}O_{2max}$ values were not reported, these results seem to support the proposal of a faster PCr recovery in those with a higher potential for aerobic metabolism. However, there are problems with this conclusion. As all participants completed exercise at the same absolute rather than relative intensity, [PCr] as a percentage of resting levels was significantly lower at the end of exercise for the controls when compared with the long-distance runners (approximately 17 vs 30%, respectively). This suggests that the pH_i, which was not reported, was also lower in the controls, because of the greater relative work output in these individuals. While it has been demonstrated that pH_i does not influence the initial rate of PCr resynthesis,[9] the results from Sahlin et al.[59] reviewed earlier, suggest that the lower pH_i of the recovering muscle in the controls would have exerted some influence over the resynthesis process before the end of the 2-minute recovery period. If this is the case, then the use of a function with a single exponential term to derive a t_c of resynthesis, given that recovery is probably already partly biphasic in at least one group, and possibly both, is inappropriate. Theoretically, this effect should be exaggerated, given that the two groups almost certainly had a significantly different end-exercise

pH_i and therefore a significantly different inhibition on PCr recovery.

Takahashi et al.[6] used ^{31}P-NMR to examine the relationship between $\dot{V}O_{2max}$ and the rate of PCr resynthesis following four different bouts of knee extension in a group of five endurance-trained runners and seven untrained controls. Mean $\dot{V}O_{2max}$ (± SEM) was 66.2 (± 2.9) and 52.0 (± 4.1) ml/kg/min respectively. As mentioned in section 8, exercise was terminated when the P_i peak was at varying levels with respect to the height of the PCr peak (light, moderate and severe exercise). A fourth exercise bout was performed and concluded when the participants reached exhaustion. Following the two more intense bouts, a significantly lower t_c, indicating faster PCr resynthesis, was found for the runners when compared with the controls ($p < 0.05$). In addition, when all of the participants were combined in a single group, there were significant correlations between $\dot{V}O_{2max}$ and the t_c following all four of the exercise intensities ($r = 0.74$, $p < 0.01$ for light, $r = 0.85$, $p < 0.01$ for moderate, $r = 0.78$, $p < 0.05$ for severe and $r = 0.71$, $p < 0.05$ for exhausting exercise). While apparently lending support to the claim that an elevated $\dot{V}O_{2max}$ results in a faster rate of PCr resynthesis, this study failed to show a cause and effect relationship between MAP and the rate of PCr resynthesis. It is reported that three of the four exercise protocols induced a fall in pH_i ($p < 0.05$) in the controls and that the runners showed a similar response to the two more intense bouts.[6] The greatest difference in mean pH_i between the two groups occurred following the most exhaustive bout: $pH_i = 6.75$ (SEM = 0.05) for the runners versus $pH_i = 6.61$ (SEM = 0.04) for the controls. Takahashi et al.[6] also used a t_c derived from a mono-exponential function to describe PCr recovery; this failed to recognise the fact that when pH_i significantly falls, as it did in this study, then PCr recovery is biphasic in nature. The problem is further compounded by the fact that Takahashi et al.[6] monitored recovery for 10 minutes, suggesting that the t_c of PCr recovery was more reflective of the slower component of recovery. If it is assumed that this component is rate-dependant on pH_i, then the significant correlations that were obtained may reflect nothing more than an increased ability of the endurance runners to restore pH_i; a known ability of individuals with an elevated $\dot{V}O_{2max}$.[24,25]

Cooke et al.[25] used ^{31}P-NMR to examine the influence of MAP on the recovery of PCr and pH_i within gastrocnemius muscle following a 2-minute bout of plantar flexion. Exercise was performed at a rate of 30 repetitions per minute against a load corresponding to 120% of a previously determined graded protocol maximum. Following a treadmill test to determine $\dot{V}O_{2max}$, 11 volunteers were placed in a high aerobic power (HAP) group and 10 in a low aerobic power (LAP) group with mean $\dot{V}O_{2max}$ values ± standard error (SE) of 64.6 ± 1.4 and 46.6 ± 1.1 ml/kg/min, HAP and LAP, respectively. The 2-minute bout of exercise depleted PCr to a similar level in the HAP (25% of resting level) when compared with the LAP (27% of resting level) group, and there was no significant difference ($p > 0.05$) in [PCr] between the two groups at any time during the subsequent 10-minute recovery period. This study was almost exactly the same as that previously conducted by the same group from which similar results had been obtained.[24] In this earlier study, individuals were either endurance trained ($n = 7$) or sedentary ($n = 7$) with mean $\dot{V}O_{2max}$ values ± SE of 67.0 ± 1.5 and 46.1 ± 1.6 ml/kg/min, respectively. Plantar flexion, at the same rate and intensity, had also been used as the mode of exercise to deplete PCr and its resynthesis over the initial 10 minutes of recovery was monitored. As in the subsequent study, PCr resynthesis showed a similar time-course in both groups.

This appears to demonstrate that when exercise is of a sufficient intensity to induce a decline in pH_i, PCr recovery is not faster in those individuals with an elevated MAP. However, for at least two reasons, this conclusion may not be correct. First, as demonstrated by Roussell et al.,[63] following exercise which induces a significant decline in pH_i there is a large variation in the rate constant of PCr

recovery. While the exact mechanism of action is not known, it appears that the low pH_i inhibits the oxidative capacity of skeletal muscle to synthesise ATP and therefore to resynthesise PCr. This variation would make it difficult to achieve statistical significance in any study that employs a relatively small subject population. Unfortunately, this is a characteristic of much of the literature that uses ^{31}P-NMR to examine this research question and the possibility therefore exists that this variation is responsible for the difficulty in establishing a relationship between MAP and the rate of PCr resynthesis following intense exercise. A differing end-exercise metabolic state could be responsible for a specific point raised by Cooke et al.[25] during a discussion of two individuals assigned to their endurance-trained group on the basis of their high aerobic power. While belonging to the same group, particular reference was made to the fact that these two individuals displayed very different PCr recovery kinetics during the first 3 minutes following exercise. Despite performing plantar flexion against an identical relative resistance during the preceding exercise bout, it remains possible that the metabolic state within the muscle at the end of exercise, and the pH_i in particular, was different in the two individuals.

The results reported by Roussell et al.[63] suggest that this may be the reason for the different PCr recovery kinetics between the individuals rather than a difference in their individual abilities to resynthesise PCr. It was noted that with a variation of this nature, it seemed little wonder that the analysis of group means showed no difference between recovery rates.[25] Alternatively, the inability to find a difference in PCr recovery rates between endurance-trained athletes and their more sedentary counterparts, may be the function of a different form of variation. $\dot{V}O_{2max}$, the variable chosen by Cooke et al.[25] to assign individuals to groups, is a measure of the maximal amount of O_2 the body can use per unit of time and is therefore a product of both oxygen transport and oxygen utilisation. This relationship can be expressed as (equation 9):

$$\dot{V}O_{2max} = \dot{Q}_{max} \times (a-\bar{v})O_{2max}$$

While there is continuing debate as to whether the factors that limit $\dot{V}O_{2max}$ during a whole body activity such as running, are centrally [cardiac output ($\dot{Q}$)] or peripherally ($a\text{-}\bar{v}O_2$) located, it seems reasonable to conclude that they will be different to those factors that limit the maximal aerobic capacity of a single calf muscle following plantar flexion exercise. The maximal aerobic capacity of a single muscle is more likely to be limited by peripheral factors such as capillary density, myoglobin levels and mitochondrial density. It is these factors that will allow ATP production to proceed at its maximal rate in an environment where the muscle is probably being supplied with all of the oxygen it can use; factors which determine whole body MAP, will almost certainly not be challenged by the oxygen demands of the small recovering muscle mass.

Further compounding this problem is the fact that as MAP is the product of both $\dot{Q}$ and $a\text{-}\bar{v}O_2$, a large variation in these two values can still yield a similar MAP and therefore result in identical group assignment. Although aerobic training responsible for elevating factors such as cardiac output and blood volume will almost certainly bring about a degree of adaptation in these peripheral components, a large variation in $\dot{Q}$ and $a\text{-}\bar{v}O_2$ may still give rise to similar levels of MAP.[87]

A variation of this nature could also be responsible for the observed difference in PCr recovery rates between the two endurance-trained individuals discussed by Cooke et al.[25] and noted above. This review to date would suggest that the initial fast phase of PCr recovery is complete well within 3 minutes following exercise, and that by this stage, the rate of PCr resynthesis has become dependent on the recovery of pH_i. It seems possible that one of these individuals had an elevated $a\text{-}\bar{v}O_2$ when compared with the other, which meant they were better able to utilise the O_2 that was being

provided to the muscle to resynthesise PCr. However, as this same individual may then have had a comparatively smaller $\dot{Q}$, the product of both of these variables would have resulted in the individuals being assigned to the same group.

With this in mind, work recently completed in this laboratory (unpublished observations) used one-legged cycling to assign individuals to either a high or low aerobic power group (HAP and LAP, respectively). As peak aerobic power during one-legged cycling has been shown to be around 75% of that which can be obtained using two-legged cycling,[88,89] this method of group assignment was used as it was considered likely to be limited by the peripheral rather than central components of aerobic metabolism. In this way it was considered more likely that the same factors that limit group assignment, would also determine the recovery of a single muscle mass when central factors such as blood flow and left ventricular output were unlikely to be limiting. Participants completed 96 seconds of leg extension against a load corresponding to 60% of maximal load at a rate of 30 repetitions per minute. At the end of exercise, which had reduced pH_i to 6.83 ± 0.1 and 6.83 ± 0.09 (HAP and LAP, respectively, $p > 0.05$), [PCr] ± standard deviation had fallen to 31.92 ± 8.82 and 29.35 ± 5.37% of resting levels in these two groups ($p > 0.05$). However, 56 seconds into passive recovery, the HAP group had a significantly greater level of PCr when compared with the LAP group (82.30 ± 7.10 *vs* 74.20 ± 3.91% of resting levels, respectively).

To our knowledge, only one published study has examined the effect of an endurance-training programme on the rate of PCr resynthesis following exercise in humans.[42] This study examined PCr resynthesis following a bout of plantar flexion which reduced PCr levels to approximately 50 to 60% of resting levels. Following 14 days of endurance training, the t_c (± standard deviation) of PCr resynthesis was significantly decreased from 25.4 ± 1.7 to 21.2 ± 1.7 seconds ($p < 0.05$). The t_c had returned to pre-exercise levels 42 days following the cessation of training (28.2 ± 2.1 seconds, $p > 0.05$ when compared with post-training values). Although this significant decline represents an increased ability to resynthesise PCr following endurance training, the intensity of the preceding exercise bout should be noted. As contraction was terminated when PCr had dropped to between 50 and 60% of resting levels, and end-exercise pH_i remained at approximately 7.0 to 7.1, a similar effect of endurance training following more intense exercise can not be assumed.

10. Conclusion

While endurance training has been associated with a faster rate of PCr resynthesis following low-intensity muscle contraction, conjecture remains as to whether high aerobic power is related to PCr recovery rate following intense exercise. There appears to be a large interindividual and inter-protocol variation in the rate constant of PCr recovery when pH_i falls significantly. This variation means it is difficult to establish a causal relationship between aerobic power and the rate of PCr resynthesis following intense exercise. Further research is therefore required to elucidate the most dominant components governing the resynthesis of PCr following intense exercise.

Acknowledgements

The authors would like to acknowledge the support of the Australian Rugby Union in funding extensive research examining this topic and the compilation of this review.

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