Influence of dietary creatine supplementation on muscle phosphocreatine kinetics during knee-extensor exercise in humans

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Running Head: Creatine loading and [PCr] kinetics
Abstract

We hypothesised that increasing skeletal muscle total creatine (Cr) content through dietary Cr supplementation would result in slower muscle [PCr] kinetics, as assessed using $^{31}$P- magnetic resonance spectroscopy, following the onset and offset of both moderate-intensity exercise (Mod) and heavy-intensity exercise (Hvy). Seven healthy males (mean ± SD, age 29 ± 6 years) completed a series of square-wave transitions to Mod and Hvy knee-extensor exercise inside the bore of a 1.5 T superconducting magnet both before and after a 5-day period of Cr loading (4 x 5 g/day of creatine monohydrate). Cr supplementation resulted in a ~ 8% increase in the resting muscle PCr/ATP ratio ($4.66 ± 0.27 \text{ vs. } 5.04 ± 0.22; P<0.05$), consistent with a significant increase in muscle total Cr content consequent to the intervention. The time constant for muscle [PCr] kinetics was increased following Cr loading for Mod exercise (Con: $15 ± 8 \text{ vs. } Cr: 25 ± 9 \text{ s; } P<0.05$) and subsequent recovery (Con: $14 ± 8 \text{ vs. } Cr: 27 ± 8 \text{ s; } P<0.05$), and for Hvy exercise (Con: $54 ± 18 \text{ vs. } Cr: 72 ± 30 \text{ s; } P<0.05$) but not subsequent recovery (Con: $41 ± 11 \text{ vs. } Cr: 44 ± 6 \text{ s}$). The magnitude of the increase in [PCr] following Cr-loading was correlated ($P<0.05$) with the extent of the slowing of the [PCr] kinetics for the moderate off-transient ($r = 0.92$) and the heavy on-transient ($r = 0.71$). These data demonstrate, for the first time in humans, that an increase in muscle [PCr] results in a slowing of [PCr] dynamics in exercise and subsequent recovery.

Key Words: [PCr] kinetics; $\dot{V}_O_2$ kinetics; respiratory control; creatine kinase; muscle energetics; $^{31}$P-MRS
Introduction

Meyer’s ‘electrical analogue’ model of respiratory control posits that the time constant (\(\tau\)) for the exponential fall in muscle phosphorylcreatine concentration ([PCr]) following the onset of muscle contractions is a function of the mitochondrial resistance and the metabolic capacitance (45). The model predicts that an increase in mitochondrial density will result in a shorter \(\tau\) (that is, faster [PCr] kinetics), and that an increase in metabolic capacitance, determined predominantly by the muscle total creatine (Cr) content, will result in a longer \(\tau\) (slower [PCr] kinetics). In keeping with this prediction, Meyer and colleagues reported that increased citrate synthase activity induced by endurance training resulted in faster [PCr] kinetics in rat muscle (49), and that depletion of the total Cr content of rat gastrocnemius muscle, achieved through 8 weeks of feeding with the Cr analogue ß-guanidinopropionic acid, resulted in a significantly faster [PCr] kinetics (46).

It is well established that muscle [PCr] and oxygen uptake (\(\dot{V}_{\text{O}_2}\)) change with similar kinetic profiles following the onset of exercise until a new steady-state is attained (3, 7, 42-44, 55-57). These data indicate that the rate of mitochondrial respiration is intimately linked, through the creatine kinase (CK) reaction, to the ATP/ADP ratio and/or the PCr/Cr ratio (1, 9, 42, 47). In an important recent study, Kindig et al. (35) reported that acute CK inhibition led to a significantly faster fall in intracellular PO2 (equivalent in this model to a faster rise in \(\dot{V}_{\text{O}_2}\)) following the onset of contraction in isolated \textit{Xenopus} myocytes. Moreover, Glancy et al. (16) demonstrated that the \(\tau\) for oxygen consumption in isolated mitochondria was linearly related to the total Cr content in the incubation medium.

Collectively, these data provide strong support for the notion that the \(\tau\) for both muscle [PCr] and \(\dot{V}_{\text{O}_2}\) conforms to a simple linear model of respiratory control, at least under the conditions of the experiments performed. In humans, however, data relating [PCr] and/or \(\dot{V}_{\text{O}_2}\) kinetics to the muscle total creatine content are sparse. Francescato et al. (15) have recently reported that inter-subject differences in resting muscle [PCr] was positively correlated (\(r=0.71\)) with the \(\tau\) for the fall in [PCr] during plantar flexion exercise in humans. However, the range of resting [PCr] values was relatively small in that study. A better test of the expected relationship between muscle total Cr content and the \(\tau\) for [PCr] during exercise would involve an intervention which alters the total Cr content relative to the control condition. In this regard, dietary supplementation with creatine monohydrate has
been demonstrated to increase muscle Cr by >20%, of which 20-30% is in the form of PCr (22). According to Meyer’s electrical analogue model, a greater muscle total Cr content consequent to dietary Cr ‘loading’ would be expected to result in slower muscle PCr and \( \dot{V}_O_2 \) kinetics. In the only study to date on this topic, Jones et al. (28) were unable to detect a significant change in the phase II pulmonary \( \dot{V}_O_2 \) kinetics following Cr loading either at moderate (i.e., below the estimated lactate threshold, LT) or heavy (i.e., >LT) intensities of cycle ergometer exercise. However, pulmonary \( \dot{V}_O_2 \) kinetics can only provide an indirect estimate of muscle [PCr] or \( \dot{V}_O_2 \) kinetics (4, 18, 56) and it is possible that such measurements were not sufficiently sensitive to detect changes in the dynamics of muscle oxidative metabolism following Cr loading.

The purpose of the present study was therefore to investigate the influence of dietary Cr supplementation on muscle [PCr] kinetics (as determined using \(^{31}\)phosphate-magnetic resonance spectroscopy; \(^{31}\)P-MRS) during knee extension exercise in humans. We hypothesised that increasing the muscle total Cr content (and thus metabolic capacitance) through creatine loading would result in slower muscle [PCr] kinetics following the onset and offset of both moderate-intensity and heavy-intensity exercise.

**Methods**

**Subjects**

Seven healthy male subjects (mean ± S.D. age 29 ± 6 years; stature 1.80 ± 0.04 m; mass 81.7 ± 7.3 kg) volunteered and gave written informed consent to participate in the study which had been approved by the local Research Ethics Committee and which conformed to the Declaration of Helsinki. The subjects were active in strength and power sports (e.g., weight-training, sprinting). The subjects were instructed to arrive at the laboratory for each of their tests in a well-hydrated state, and having consumed no food or caffeine during the previous 3 hours, and no alcohol during the previous 24 hours.

**Experimental Protocol**

The single-legged knee-extension ergometer and associated exercise tests have been described in detail elsewhere (29). Briefly, the exercise tests were conducted in the prone position with
the subjects positioned inside a whole body MRI system. A 6cm $^{31}$P transmit /receive surface coil was placed within the subject bed and the subject asked to lie upon it such that the coil was centred over the quadriceps muscle of the right leg. Subjects were then secured to the ergometer bed with Velcro straps at the thigh, buttocks and lower back to minimise extraneous movement during the protocol. The right foot was fastened to a padded foot brace, which was connected to the ergometer load basket using a rope and pulley system. Exercise was performed at a rate of 40 repetitions per minute with the subjects lifting and lowering the mass over a distance of ~ 0.22 m in accordance with a visual cue projected onto the front wall of the scanner room. The contraction phase of the knee extensors and the interrogation of the quadriceps by $^{31}$P-MRS were synchronised such that spectra were acquired immediately prior to the concentric phase of the muscle contraction.

Initially, each subject completed an incremental exercise test to volitional exhaustion. Following a 2-min period of rest, the subjects commenced knee-extension exercise against an initial basket load of 0.5 kg. Thereafter, the basket load was increased by 0.5 kg at the end of each minute until the subjects were no longer able to maintain the required frequency of 40 repetitions per minute. The subjects received strong verbal encouragement to continue for as long as possible while maintaining appropriate form (i.e. smooth, continuous movement over the full range of motion). To determine the intracellular threshold (IT), piecewise linear regression was used as described previously (29). Briefly, different two-line combinations were fitted to the $[\text{Pi}/[\text{PCr}]$-work rate and pH-work rate relationships until the lowest sum of squared residuals was identified. The point at which this particular two-line combination intersected was accepted as the IT.

Subsequently, the subjects completed a series of ‘square-wave’ exercise tests of moderate intensity (75% of the IT, corresponding to approximately 40% of the peak work rate recorded during the incremental test) and heavy-intensity (50% of the difference between the IT and the peak work rate, corresponding to approximately 80% peak work rate) exercise both before and after a 5-10 day period of dietary Cr supplementation. The exercise protocol consisted of a 2-min resting baseline period followed by two bouts of moderate-intensity exercise and one bout of heavy-intensity exercise. The exercise bouts were all of 6-min duration and were separated by 6-min of resting recovery. Pilot experiments demonstrated that the performance of prior moderate-intensity exercise did not alter the [PCr] response dynamics during subsequent heavy-intensity exercise. This protocol was repeated twice both before and after Cr loading.
such that a total of 4 moderate-intensity and 2 heavy-intensity exercise bouts were completed before and after the Cr loading intervention.

During the supplementation period participants were prescribed a $4 \times 5 \text{g} \cdot \text{d}^{-1}$ dose of creatine monohydrate. The Cr loading regimen applied in this study has been previously shown to be effective in significantly increasing the intramuscular PCr content (22, 24). Participants were instructed to consume the supplements, dissolved in a warm drink, at regular intervals throughout the day with a meal where possible and to avoid simultaneous caffeine intake in order to aid Cr intake. Participants were also given a journal for each supplementation period and were asked to log the times supplements were ingested. Self-reported compliance to supplementation across the group was 100%.

MRS Measurements

MRS was performed in the Peninsula Magnetic Resonance Research Centre, Exeter, using a 1.5 T superconducting MR scanner (Intera, Philips, Netherlands). Initially, fast field echo images were acquired in order to determine that the muscle was positioned correctly relative to the coil. This was aided by placing cod liver oil capsules, which yield high intensity signal points within the image, adjacent to the coil allowing its orientation relative to the muscle volume under examination to be assessed. A number of pre-acquisition steps were carried out in order to optimize the signal from the muscle under investigation. An automatic shimming protocol was then undertaken within a volume that defined the quadriceps muscle and matching and tuning of the coil was then performed. To ensure that the examined muscle was consistently at the same point relative to the coil during exercise, the subject was visually queued via a display consisting of two vertical bars, one which moved at a constant rate with a frequency of 0.67 Hz and one which monitored their foot movement via a sensor present within the pulley to which they were connected. Thus, the subject endeavoured to match the movements of these two bars. The work done by the subject was recorded via a non-magnetic strain gauge present within the pulley mechanism, enabling work-rate to be calculated.

Prior to and during exercise, data were acquired every 1.5 seconds, with a spectral width of 1500 Hz, and 1K data points. Phase cycling with 4 phase cycles was employed, leading to a spectra being acquired every 6 s. The subsequent spectra were quantified via peak fitting, assuming prior knowledge, using the jMRUI (version 2) software package employing the
AMARES fitting algorithm (64). Spectra were fitted assuming the presence of the following peaks: P$_i$, phosphodiester, PCr, $\alpha$-ATP (2 peaks, amplitude ratio 1:1), $\gamma$-ATP (2 peaks, amplitude ratio 1:1) and $\beta$-ATP (3 peaks, amplitude ratio 1:2:1). In all cases, relative amplitudes recorded during exercise were corrected for partial saturation by obtaining a baseline spectra prior to exercise with long repetition time (TR=20s) in which the relative unsaturated peak amplitudes could be determined.

Intracellular pH was calculated using the chemical shift of the P$_i$ spectral peak relative to the PCr peak (60). Resting and end-exercise values of PCr, P$_i$, and pH were calculated over the last 30 s of the rest or exercise period. The absolute muscle [PCr] values were estimated using the PCr/ATP ratio and assuming an [ATP] of 8.2 mM (33, 61). ADP concentration and the free energy of ATP hydrolysis ($\Delta G_{ATP}$) were calculated as described by Kemp et al (34).

**Modelling Procedures**

To enhance the signal-to-noise properties and therefore the underlying features of the [PCr] response profile before kinetic parameter estimation, each subject's repeat constant work exercise transitions were time-aligned to the onset of exercise ($t = 0$ s) and averaged, yielding a single [PCr] response with a sample resolution of 6 s. Using the resulting averaged constant work response profile, each subject's PCr responses were normalized relative to the previous steady-state baseline, using the average PCr value during the 2-min rest or exercise period for determination of the onset and offset kinetics, respectively.

The [PCr] responses were subsequently modelled using non-linear least-squares regression techniques. We used a similar procedure to that of Rossiter et al. (57). Briefly, an exponential function of the form:

$$\Delta[PCr](t) = [PCr]_{ss} \cdot (1-e^{-t/\tau})$$

[Eqn. 1]

where $\Delta[PCr]_{ss}$ is the projected asymptotic value, and $\tau$ is the time constant of the response, was fit to the data. The first fit contained only the first 60 s of exercise data but the fitting window was then increased iteratively until there was a clear departure of the measured data from the model fit, as judged from visual inspection of a plot of the residuals. In this way, the
best-fit exponential for the fundamental component of the response was established. The magnitude of any possible [PCr] slow component was then calculated as the difference between the asymptotic amplitude of the fundamental [PCr] response and the average [PCr] measured over the last 30 s of exercise or rest for that condition. In order to account for possible differences in [PCr] amplitude between conditions, the rate of PCr consumption following the onset of exercise or recovery was calculated as the amplitude of the [PCr] change in the fundamental phase of the response divided by the time constant (A/τ). In addition, the half-time (T_50) of the response from rest to the end of exercise and from the end of exercise to the end of recovery was computed from the raw data. We elected not to formally model the ADP or ∆G_ATP responses due to the numerous assumptions (and thus potential for error) involved in their calculation and because the responses were not always clearly exponential.

Statistics

Differences in the parameters of the [PCr] kinetics between the two conditions were examined using paired t-tests. Pearson product moment correlation coefficients were used to explore the relationships between changes in the baseline [PCr] following Cr loading and the [PCr] time constant during moderate-intensity and heavy-intensity exercise. Significance was accepted when P<0.05. Values are expressed as mean ± SD.

Results

Dietary Cr supplementation resulted in a ~ 8% increase in the resting muscle PCr/ATP ratio (4.66 ± 0.27 vs. 5.04 ± 0.22; P<0.05), consistent with a significant increase in muscle total Cr content consequent to the intervention. The resting [PCr]/[ATP] ratio and the estimated resting [PCr] (~ 38 mM) in our study was at the upper end of the range reported in the literature using similar techniques (33).

Moderate-intensity exercise: [PCr] on- and off-kinetics

Muscle pH was not significantly different between the control and Cr-loaded conditions either during the pre-exercise baseline (CON: 7.06 ± 0.03 vs. CRE: 7.06 ± 0.02), at the end of exercise (CON: 7.08 ± 0.01 vs. CRE: 7.07 ± 0.02), or at the end of the recovery period (CON: 7.05 ± 0.03 vs. CRE: 7.03 ± 0.03), (Figure 1). Similarly, the Pi/PCr ratio was not different.
between the conditions at baseline (CON: 0.11 ± 0.02 vs. CRE: 0.10 ± 0.02), at the end of exercise (CON: 0.19 ± 0.02 vs. CRE: 0.16 ± 0.03), or at the end of the recovery period (CON: 0.11 ± 0.02 vs. CRE: 0.10 ± 0.02). The resting [Pi] was not significantly altered by Cr-loading.

The [PCr] kinetics during moderate exercise and subsequent recovery are reported in Table 1 and are illustrated for the entire group in Figure 2. The [PCr] was significantly greater during the resting baseline period prior to the onset of exercise in the Cr-loaded condition compared to the control condition. However, the magnitude of the fall in [PCr] from rest to the steady-state was not significantly different between the conditions such that the [PCr] remained significantly higher in the Cr-loaded state throughout exercise (Figure 2). Importantly, the τ (CON: 15 ± 8 vs. CRE: 25 ± 9 s; \( P < 0.05 \)) and the \( T_{50} \) (CON: 11 ± 6 vs. CRE: 17 ± 7 s; \( P < 0.05 \)) were significantly increased by Cr loading. Moreover, the initial rate of PCr degradation was significantly less following Cr loading (CON: 0.40 ± 0.14 vs. CRE: 0.18 ± 0.05 mM/s; \( P < 0.05 \)).

The [PCr] remained higher across the off-transient in the Cr-loaded condition compared to control (Figure 2). Like the on-transient, there was no difference in the amplitude of the [PCr] recovery between the conditions (Table 1). However, also like the on-transient, the [PCr] kinetics were significantly slower following Cr-loading when expressed as the τ (CON: 14 ± 8 vs. CRE: 27 ± 8 s; \( P < 0.05 \)), \( T_{50} \) (CON: 10 ± 6 vs. CRE: 18 ± 6 s; \( P < 0.05 \)), or as the \( \Lambda/\tau \) (CON: 0.36 ± 0.18 vs. CRE: 0.13 ± 0.07 mM/s; \( P < 0.05 \)).

Heavy-intensity exercise: [PCr] on- and off-kinetics

Muscle pH was not significantly different between the control and Cr-loaded conditions either during the pre-exercise baseline (CON: 7.06 ± 0.04 vs. CRE: 7.04 ± 0.02), at the end of exercise (CON: 6.95 ± 0.08 vs. CRE: 6.87 ± 0.13; \( P = 0.06 \)), or at the end of the recovery period (CON: 6.97 ± 0.04 vs. CRE: 6.88 ± 0.13), but it was significantly lower over the first 60 s of recovery following Cr loading (\( P < 0.05 \); Figure 1). Similarly, the Pi/PCr ratio was not different between the conditions at baseline (CON: 0.10 ± 0.02 vs. CRE: 0.11 ± 0.03), at the end of exercise (CON: 1.56 ± 0.79 vs. CRE: 1.77 ± 0.78), or at the end of the recovery period (CON: 0.06 ± 0.05 vs. CRE: 0.05 ± 0.02). The resting [Pi] was also not significantly altered by Cr-loading.
The [PCr] kinetics during high-intensity exercise and subsequent recovery are reported in Table 2 and the group mean response is shown in Figure 3. The [PCr] was significantly greater during the resting baseline period prior to the onset of exercise in the Cr-loaded condition compared to the control condition. As for moderate exercise, however, the magnitude of the fall in [PCr] from rest to the steady-state was not significantly different between the conditions such that the [PCr] remained significantly higher in the Cr-loaded state throughout exercise (Figure 3). The $\tau$ (CON: 54 ± 18 vs. CRE: 72 ± 30 s; $P<0.05$) and the $A/\tau$ (CON: 0.47 ± 0.14 vs. CRE: 0.31 ± 0.09 mM/s; $P<0.05$) both indicated a significant slowing of [PCr] kinetics following Cr loading.

The [PCr] remained higher across the off-transient in the Cr-loaded condition compared to control (Figure 3). There was no difference in the amplitude of the [PCr] recovery between the conditions and [PCr] was significantly greater at the end of the 6-min recovery period in the Cr-loaded condition (Table 2). In contrast to the slowing of the [PCr] kinetics noted for the heavy-intensity exercise on-transient and also for both the moderate-intensity exercise on- and off-transients, there was no significant difference in the [PCr] kinetics between the control and Cr-loaded conditions either when expressed as $\tau$ (CON: 41 ± 11 vs. CRE: 44 ± 6 s) or as $A/\tau$ (CON: 0.50 ± 0.10 vs. CRE: 0.45 ± 0.18 mM/s).

Figure 4 shows the group mean changes in muscle [PCr] kinetics following Cr loading. In this schematic, the responses are expressed relative to the change in [PCr] from baseline to end-exercise or end-recovery, enabling clearer visualisation of the generally slower response in the Cr-loaded condition.

*Relationships between the change in [PCr] and the change in $\tau$*

There were positive correlations between the magnitude of the increase in [PCr] following Cr-loading at baseline and the extent of the associated slowing of the [PCr] kinetics: moderate exercise on-transient, $r = 0.60$; moderate exercise off-transient, $r = 0.92$ ($P<0.05$); heavy-intensity exercise on-transient, $r = 0.71$ ($P<0.05$); and heavy-intensity exercise off-transient, $r = 0.39$. 
Influence of creatine loading on ADP and $\Delta G_{ATP}$

The dynamics of free ADP during moderate and heavy exercise and subsequent recovery, before and after Cr-loading is shown in Figure 5. [ADP] was blunted following Cr loading. Although we did not formally model the ADP dynamics, the response appeared to be exponential and to adapt with a similar time course to [PCr] during the moderate on- and off-transients (Figure 5A). During heavy exercise, however, the increase in [ADP] with time was essentially linear, whereas, in recovery, the dynamics appeared to be exponential (Figure 5B). The $\Delta G_{ATP}$ response profiles are shown in Figure 6. The $\Delta G_{ATP}$ was lower following Cr-loading and had similar temporal profiles to [PCr] during moderate and heavy exercise.

Discussion

The principal novel finding of this investigation was that dietary Cr supplementation resulted in a significant slowing of muscle [PCr] kinetics during moderate-intensity exercise and subsequent recovery and also during heavy-intensity exercise. These results were consistent with our hypothesis and support the linear model of respiratory control originally proposed by Meyer (45, 46). Although a number of studies using isolated mitochondria or animal models have provided evidence in favour of the notion that muscle [PCr] (and/or $\dot{V}_O2$) kinetics are related to the total muscle Cr content (16, 35, 46), to our knowledge, the present data are the first to demonstrate that an increase in muscle [PCr], and thus an increase in metabolic capacitance (45), is associated with a slowing of muscle [PCr] kinetics during constant-load sub-maximal exercise and subsequent recovery in human subjects. The data are also consistent with the computer modelling predictions of Korzeniewski and Zoladz (37).

The dietary Cr supplementation regimen that we employed in the present study has been repeatedly demonstrated to increase the muscle total Cr content by ~20% and the muscle PCr content by ~10-20% (8, 12, 22, 24). In the present study, we used $^{31}$P-MRS to estimate the change in muscle [PCr] brought about by dietary Cr supplementation using the values for [PCr]/[ATP] reported in the literature (33) and with the reasonable assumption that muscle [ATP] was not altered by the intervention (8, 22, 24). It has been demonstrated that $^{31}$P-MRS produces similar estimates of energy metabolites, including the [PCr]/[ATP] ratio, both at rest and during exercise, as biochemical determinations derived from muscle biopsy samples (2). In the present study, dietary Cr supplementation resulted in an 8-10% increase in muscle [PCr],
with all subjects showing an increase (range: 5-14%). These data are consistent with previous investigations which reported similar increases in muscle [PCr] using either muscle biopsy (8, 12, 22, 24) or $^{31}$P-MRS (53, 58, 62) techniques, and indicate that the Cr loading intervention successfully enhanced muscle PCr (and presumably Cr) concentrations.

We tested the hypothesis that an increase in muscle total Cr content (implied by the measured increase in muscle [PCr]/[ATP] following Cr loading) would result in a slowing of the muscle [PCr] kinetics following both the onset and offset of constant-work-rate sub-maximal exercise. Although some previous studies have also used $^{31}$P-MRS to study the muscle metabolic consequences of Cr loading, these investigations have typically focused on repeated, short-duration, high-intensity exercise bouts, often performed to exhaustion (53, 59, 62, 69), which are not optimal for studying changes in [PCr] kinetics. Consistent with our hypothesis, muscle [PCr] kinetics was indeed significantly slowed by Cr loading for moderate-intensity exercise and subsequent recovery, and also during heavy-intensity exercise (but not subsequent recovery), as summarised in Figure 4. These data are consistent with the linear model of respiratory control proposed by Meyer (45, 46). This can be envisaged as an electrical circuit in which the capacitance is linked to the CK reaction (with PCr being analogous to the stored charge on a capacitor) and the resistor is a function of the number and properties of the mitochondria (45). An increase in mitochondrial density and/or oxidative enzyme activity as a consequence of endurance training, for example, would be expected to result in a speeding of muscle [PCr] (and $\dot{V}_{O_2}$) kinetics both during exercise and recovery. This prediction has been confirmed by Paganini et al. (49) who demonstrated that increased citrate synthase activity induced by endurance training resulted in faster [PCr] kinetics in rat muscle, and also by Glancy et al. (16) who recently reported that increasing the content of isolated mitochondria in an incubation medium was associated with linear reductions in the $\tau$ for $O_2$ consumption. In humans, it is well known that $\dot{V}_{O_2}$ kinetics are faster (5, 6, 36, 39, 50) and that the extent of PCr splitting for a given work rate is spared (23, 29, 70) in endurance-trained subjects.

Meyer’s ‘electrical analogue’ model also predicts that the metabolic capacitance will be linearly related to the $\tau$ for muscle [PCr] (and $\dot{V}_{O_2}$) kinetics. Our results, which show that Cr loading increased the [PCr] $\tau$, are consistent with other recent studies which indicate a critical role for the CK reaction in the regulation of mitochondrial respiration. Roman et al. (54) and Gustafson & van Beek (20) have reported a faster activation of oxidative phosphorylation following CK deletion in mice. Moreover, Kindig et al. (35) demonstrated that acute CK
inhibition resulted in a significantly faster fall in intracellular PO2 following the onset of contractions in isolated *Xenopus* myocytes which is equivalent in this model to a faster rise in $V_02$. These authors interpreted their data to indicate that cytosolic CK serves as temporal buffer to a decreased ATP/ADP following the onset of contractions, thus blunting changes in [ADP] and the phosphorylation potential and slowing mitochondrial activation. In a recent study, Glancy et al. (16) reported a linear relationship between the $\tau$ for O$_2$ consumption kinetics and the total Cr concentration in isolated rat skeletal muscle mitochondria. In humans, Francescato et al. (15) have shown that the $\tau$ for muscle [PCr] kinetics was significantly correlated ($r = 0.71$) with the initial (resting) [PCr]. We were unable to confirm this latter relationship in the present study ($r = 0.09-0.46$), possibly as a consequence of the relatively small sample size. However, the increased muscle [PCr] observed following Cr loading was positively correlated with the lengthening of the $\tau$ for [PCr] kinetics both during exercise and recovery, with the relationships being statistically significant for the moderate off-transient and the heavy on-transient.

The slowing of the [PCr] kinetics we observed following Cr loading was somewhat greater than might have been predicted. For example, assuming a linear system, a 5-15% increase in muscle total Cr would be expected to result in a proportionately similar increase in the [PCr] $\tau$. For the heavy-intensity bout, our results were quite close to the expected values (i.e., $\tau$ increased by 33% in the on-transient and by 12% in the off-transient following Cr loading). However, for the moderate-intensity bout, $\tau$ was substantially greater following Cr loading (by 67% in the on-transient and by 93% in the off-transient). We are unable to explain this greater than anticipated slowing of the [PCr] kinetics during moderate-intensity exercise and subsequent recovery with Cr ingestion. However, it is important to note that experiments with human volunteers are subject to both biological and technical variability. While we attempted to enhance the signal/noise ratio by having our subjects perform a number of like-transitions, such that the 95% confidence intervals for the estimation of $\tau$ averaged ± 4-7 s, it remains possible that ‘noise’ in the data was at least partly responsible for the larger-than-expected effects. By the same token, the lack of significant difference in the $\tau$ for [PCr] kinetics in the recovery from heavy-intensity exercise, despite the 12% slowing observed with Cr loading, might also be related to intra-subject or inter-subject variability in the measurement of [PCr].

The model of respiratory control proposed by Meyer (45) did not consider the effects of alterations in pH, and it is known that changes in the equilibrium of the CK reaction such as
might occur during and following high-intensity exercise, can complicate both the description and the interpretation of muscle [PCr] dynamics (10, 21, 25, 40, 41). In this regard, it is of interest that the fall in pH during heavy-intensity exercise tended to be greater in the Cr-loaded condition compared to the control condition ($P=0.06$), possibly as a consequence of the slower rate of PCr hydrolysis and presumably oxidative phosphorylation (but see later discussion) and the requirement for a compensatory increase in glycolytic ATP supply. To what extent the lower pH during heavy-intensity exercise (and over the first 60 s of recovery; Figure 1) with Cr loading independently influenced the [PCr] kinetics is difficult to establish.

In the control condition of the present study, the $\tau$ was appreciably longer for heavy-intensity exercise where pH was significantly reduced, than for moderate-intensity exercise where it was unchanged (Figures 1 and 4). Slower [PCr] kinetics at higher compared to lower intensities of exercise can be interpreted to signify the existence of a respiratory control system with additional complexity (46). Binzoni et al. (7) reported that the kinetics of PCr hydrolysis were not significantly different between five ‘aerobic’ exercise bouts in which muscle pH was not perturbed. Rossiter et al. (56) found that [PCr] kinetics were somewhat faster for moderate-intensity exercise ($\tau$, ~33s) compared to heavy-intensity exercise ($\tau$, ~38 s) but this difference was not statistically significant. On the other hand, several groups have reported that [PCr] kinetics were significantly slower when muscle pH was reduced (26, 41, 63, 66). In addition to the putative effects of pH on respiratory control, the longer [PCr] $\tau$ at higher work rates, which is often but not always mirrored in pulmonary $\dot{V}O_2$ kinetics (51), might be related to the recruitment of type II muscle fibres with inherently slow kinetics (11, 13, 32).

Although our data are consistent with several earlier studies (26, 41, 63, 66), the magnitude of the slowing of [PCr] kinetics at higher compared with lower work rates in the present study was greater than might have been expected. The $\tau$ value for moderate exercise in the control condition of the present study (~15 s) was somewhat smaller than we (32) and others (3, 4, 55) have reported previously (~23-35 s), but similar to the values that have been considered to be realistic for moderate exercise (52). On the other hand, the $\tau$ value for heavy exercise in the control condition of the present study (~55 s) was similar to or somewhat greater than we (29, 30, 32) and others (14, 56, 57) have reported previously (38-54 s). The explanation for the substantial slowing of [PCr] kinetics between moderate and heavy exercise in the present study and also in an earlier study from our laboratory (32) is obscure but might be related to our experimental protocol which involves unilateral concentric/eccentric knee extension exercise,
potentially resulting in high muscle tension development during high-intensity exercise and consequent impairments to muscle blood flow and oxygenation (30). The finding that the [PCr] kinetics was faster in the off-transient than the on-transient for the heavy exercise condition is consistent with this interpretation. The slower [PCr] kinetics we observed for the heavy off-transient compared to the moderate off-transient (~41 vs. ~ 15 s) differs from two previous studies (13, 56). For example, Forbes et al. (13) recently reported that the τ for [PCr] was similar in human triceps surae muscle in the recovery from low and high intensity plantar flexion exercise. Explanations for the differences between studies might include a greater heterogeneity of oxidative capacity in the muscle fibers recruited during knee-extension as compared to plantar flexion exercise, the fact that the subjects in our study practiced sprint or strength training, and the higher exercise intensity in our study compared to previous studies as reflected in a greater reduction in [PCr].

It has been proposed that an increased capacity for PCr re-synthesis during recovery is the principal mechanism by which Cr ingestion might improve performance during repeated high-intensity exercise (8, 19, 22, 27). At face value, the tendency for the muscle [PCr] kinetics to be slower following Cr ingestion in the present study would seem to contradict this notion. However, the higher muscle [PCr] that was present throughout exercise and recovery in the Cr-loaded condition might be expected to predispose to enhanced exercise tolerance (19, 31, 58). Moreover, the heavy-intensity exercise bout completed in the present study was ‘sub-maximal’ in that our subjects did not continue until exhaustion. There is evidence that the initial rate of PCr re-synthesis depends upon the magnitude of muscle PCr utilisation (and of free Cr accumulation). For example, Smith et al. (59) reported that Cr loading reduced the initial rate of PCr re-synthesis and increased the [PCr] τ following a brief period of high-intensity exercise (consistent with the results of the present study); however, following exhaustive exercise, the initial rate of PCr re-synthesis was greater and the [PCr] τ was unchanged in the Cr-loaded condition. Similarly, Kreis et al. (38) have shown that, following Cr ingestion, PCr recovery is slower (as indicated by the PCr rate constant), but that more PCr is re-synthesised per unit time when muscle [PCr] is fully depleted. These data are consistent with evidence that the PCr/Cr ratio influences the rate of mitochondrial respiration (65). The results of the present study therefore do not contradict the view that Cr supplementation has the potential to enhance exercise performance during repeated bouts of high-intensity exercise.
It has been demonstrated that muscle [PCr] and $\dot{V}_O_2$ change with similar kinetic profiles following the onset and cessation of exercise (3, 7, 42-44, 56, 57). These data imply that the majority of the delay in the attainment of a steady-state matching between oxidative supply and demand is linked to the rate of cytosolic ATP hydrolysis and the CK reaction (17, 51, 67). That being the case, the present results and the work of Glancy et al. (16) predict that dietary Cr supplementation should result in a slowing of the phase II pulmonary $\dot{V}_O_2$ kinetics (which reflect the fundamental component rise in muscle $O_2$ uptake following the onset of exercise to within $\pm$ 10%; 4, 18). To our knowledge, only one previous study has investigated the influence of Cr loading on pulmonary $\dot{V}_O_2$ kinetics (28). In that study, which utilised a large number of like-transitions to reduce breath-to-breath noise to a minimum, no effect on the phase II $\tau$ value was reported either for moderate-intensity or heavy-intensity cycle ergometer exercise. Interestingly, however, the $\tau$ was increased by $\sim$10% and $\sim$4% for moderate-intensity and heavy-intensity exercise, respectively. It is possible that inter-subject variability in the changes to muscle Cr content in response to dietary Cr supplementation (8, 12), which was not directly assessed by Jones et al. (28), precluded the attainment of statistical significance. In the same study, the steady-state $\dot{V}_O_2$ was not different following Cr loading for moderate-intensity exercise, consistent with the similar $\Delta$ [PCr] observed in the present study. However, Jones et al. (28) reported that both the fundamental component and end-exercise $\dot{V}_O_2$ values were slightly (3-4%) but significantly reduced for heavy-intensity exercise, results which were not mirrored in $\Delta$ [PCr] in the present study. Glancy et al. (16) have reported that the steady-state mitochondrial $O_2$ consumption was not changed when the total Cr level in the incubation medium was altered. It is possible either that the small differences in $\dot{V}_O_2$ reported earlier were not physiologically meaningful or that differences in exercise modality (including exercise intensity, contraction regimen, and motor unit recruitment patterns) can account for the difference between the two studies.

An alternative interpretation is that $\dot{V}_O_2$ kinetics were not, in fact, altered by Cr loading despite muscle [PCr] kinetics being slowed. On the basis of computer modelling studies, Korzeniewski and Zoladz (37) have suggested that $\dot{V}_O_2$ kinetics is near-linearly proportional to, and that respiratory control is therefore more closely related to, the absolute amount of PCr hydrolysed during a rest-to-exercise transition (i.e. the $\Delta$[PCr]). According to this model, a similar $\Delta$[PCr] following Cr loading (as was observed in the present study) would be expected to result in unchanged $\dot{V}_O_2$ kinetics. The present data therefore do not refute the parallel-activation model proposed by Korzeniewski and Zoladz (37).
We did not measure \( \dot{V}_O_2 \) kinetics in the present study. However, it is informative to consider the response profiles of other putative regulators of oxidative phosphorylation (at the different exercise intensities before and after Cr loading in relation to the known muscle [PCr] kinetics which have been shown to be generally similar to the \( \dot{V}_O_2 \) kinetics (3, 7, 42-44, 55-57). Models of respiratory control include substrate control in a linear fashion through changes in PCr or Cr (see earlier discussion), in a hyperbolic fashion through changes in ADP, and thermodynamic control through changes in ATP/ADP or the cytosolic free energy of ATP hydrolysis (\( \Delta G_{ATP} \), (1, 9, 40, 42, 45, 47, 48, 65, 68). The ADP response during both exercise and recovery was blunted following Cr loading as would be expected if the CK reaction temporally buffers changes to the ATP-to-ADP concentration ratio across metabolic transients (15, 16, 35, 45, 46). During moderate exercise, ADP kinetics was apparently exponential and displayed a similar time course to [PCr] kinetics (steady state attained in ~ 60 s). During heavy exercise and subsequent recovery, however, the ADP response exhibited a striking asymmetry: in the on-transient, ADP increased linearly with time (in contrast to the [PCr] response) whereas, in the off-transient, ADP returned to baseline in an exponential fashion that was faster than the recovery of [PCr]. Similar data have been reported previously by Yoshida et al. (70). The temporal characteristics of the ADP response imply that any control of mitochondrial respiration by ADP must operate in a complex fashion that is not first-order, as was also concluded by Barstow et al. (3). On the other hand, \( \Delta G_{ATP} \) exhibited similar temporal response profiles to [PCr] during both the on- and off-transients of moderate and heavy exercise. Moreover, the \( \Delta G_{ATP} \) kinetics appeared to be slower following Cr loading. Considering the limitations to data interpretation posed by the assumptions inherent in the estimation of the pertinent variables, we are unable to distinguish between mechanisms of respiratory control that feature substrate control through changes in [PCr] or thermodynamic control through changes in \( \Delta G_{ATP} \) since both responded in the predicted fashion both before and after Cr loading.

**Perspectives and Significance**

In this study, we have shown that dietary Cr supplementation, which resulted in an ~8% increase in the muscle PCr/ATP ratio, resulted in a significant slowing of muscle [PCr] kinetics in the transition from rest to both moderate-intensity and heavy-intensity exercise and also in the transition from moderate-intensity exercise to rest. To our knowledge, these data are the
first to demonstrate, in humans, that an intervention which alters muscle [PCr] results in a corresponding effect on the \( \tau \) for [PCr] dynamics in exercise and subsequent recovery. These data are consistent with other studies which implicate the CK reaction as a critical component in the regulation of oxidative phosphorylation.
References


42. **Mahler M.** First-order kinetics of muscle oxygen consumption, and an equivalent proportionality between $QO_2$ and phosphorylcreatine level. Implications for the control of respiration. *J Gen Physiol* 86: 135-165, 1985.


Figure Legends

Figure 1: Group mean pH response to moderate-intensity and heavy-intensity exercise (Panel A) and subsequent recovery (Panel B). The responses in the control condition are shown in the filled triangles (moderate) or filled circles (heavy) and the responses following 5 days of dietary creatine supplementation are shown in the open triangles (moderate) or open circles (heavy). The dashed vertical lines mark the transition from rest to exercise (Panel A) and from exercise to rest (Panel B). For clarity, the standard error is shown at 30 s intervals.

Figure 2: Group mean absolute phosphocreatine concentration ([PCr]; mM) response to moderate-intensity exercise (Panel A) and subsequent recovery (Panel B). The responses in the control condition are shown in the filled circles and the responses following 5 days of dietary creatine supplementation are shown in the open circles. The dashed vertical lines mark the transition from rest to exercise (Panel A) and from exercise to rest (Panel B). Note the elevated muscle [PCr] at rest and throughout exercise and recovery following creatine loading. Note also the slower muscle [PCr] kinetics both in the on-transient and the off-transient following Cr loading. For clarity, the standard error is shown at 30 s intervals.

Figure 3: Group mean absolute phosphocreatine concentration ([PCr]; mM) response to heavy-intensity exercise (Panel A) and subsequent recovery (Panel B). The responses in the control condition are shown in the filled circles and the responses following 5 days of dietary creatine supplementation are shown in the open circles. The dashed vertical lines mark the transition from rest to exercise (Panel A) and from exercise to rest (Panel B). Note the elevated muscle [PCr] at rest and throughout exercise and recovery following creatine loading. Note also the slower muscle [PCr] kinetics in the on-transient but not the off-transient following creatine loading. For clarity, the standard error is shown at 30 s intervals.

Figure 4: Schematic illustration of the effects of dietary creatine supplementation on muscle [PCr] kinetics when the total change in [PCr] from rest to the end of exercise or vice-versa is normalised. Panel A represents moderate-intensity exercise; Panel B represents the recovery from moderate-intensity exercise; Panel C represents heavy-intensity exercise; and Panel D represents the recovery from heavy-intensity exercise. The mean model parameters (Tables 1 and 2) have been used to derive the muscle [PCr] responses in the control condition (solid lines) and in the creatine-loaded condition (dashed line). Notice that the [PCr] response is generally
slower following creatine loading. Notice also that the [PCr] kinetics are slower for heavy-intensity exercise and recovery (Panels C and D) than for moderate-intensity exercise and recovery (Panels A and B).

*Figure 5:* Group mean ADP response to moderate-intensity and heavy-intensity exercise (Panel A) and subsequent recovery (Panel B). The responses in the control condition are shown in the filled triangles (moderate) or filled circles (heavy) and the responses following 5 days of dietary creatine supplementation are shown in the open triangles (moderate) or open circles (heavy). The dashed vertical lines mark the transition from rest to exercise (Panel A) and from exercise to rest (Panel B). For clarity, the standard error is shown at 30 s intervals.

*Figure 6:* Group mean $\Delta G_{ATP}$ response to moderate-intensity and heavy-intensity exercise (Panel A) and subsequent recovery (Panel B). The responses in the control condition are shown in the filled triangles (moderate) or filled circles (heavy) and the responses following 5 days of dietary creatine supplementation are shown in the open triangles (moderate) or open circles (heavy). The dashed vertical lines mark the transition from rest to exercise (Panel A) and from exercise to rest (Panel B). For clarity, the standard error is shown at 30 s intervals.
Table 1: Mean ± SD muscle [PCr] dynamics following the onset of, and in the recovery from, moderate-intensity exercise

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Creatine</th>
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<tbody>
<tr>
<td><strong>Exercise</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline [PCr] (mM)</td>
<td>39.3 ± 3.1</td>
<td>42.9 ± 3.2 *</td>
</tr>
<tr>
<td>Time Constant (s)</td>
<td>15 ± 8</td>
<td>25 ± 9 *</td>
</tr>
<tr>
<td>[PCr] Amplitude (mM)</td>
<td>5.8 ± 1.1</td>
<td>4.4 ± 0.8</td>
</tr>
<tr>
<td>Amplitude/Time Constant (mM/s)</td>
<td>0.40 ± 0.14</td>
<td>0.18 ± 0.05 *</td>
</tr>
<tr>
<td>End-Exercise [PCr] (mM)</td>
<td>33.4 ± 2.5</td>
<td>38.5 ± 3.9 *</td>
</tr>
<tr>
<td><strong>Recovery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline [PCr] (mM)</td>
<td>33.4 ± 2.5</td>
<td>38.5 ± 3.9 *</td>
</tr>
<tr>
<td>Time Constant (s)</td>
<td>14 ± 8</td>
<td>27 ± 8 *</td>
</tr>
<tr>
<td>[PCr] Amplitude (mM)</td>
<td>4.9 ± 1.2</td>
<td>3.4 ± 0.7</td>
</tr>
<tr>
<td>Amplitude/Time Constant (mM/s)</td>
<td>0.36 ± 0.18</td>
<td>0.13 ± 0.07 *</td>
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<tr>
<td>End-Recovery [PCr] (mM)</td>
<td>38.3 ± 1.8</td>
<td>41.9 ± 4.1</td>
</tr>
</tbody>
</table>

* = P<0.05.
Table 2: Mean ± SD muscle [PCr] dynamics following the onset of, and in the recovery from, heavy-intensity exercise

<table>
<thead>
<tr>
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<th>Creatine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exercise</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline [PCr] (mM)</td>
<td>37.3 ± 2.9</td>
<td>42.0 ± 3.7*</td>
</tr>
<tr>
<td>Time Constant (s)</td>
<td>54 ± 18</td>
<td>72 ± 30 *</td>
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<tr>
<td>[PCr] Amplitude (mM)</td>
<td>20.1 ± 7.2</td>
<td>21.0 ± 8.8</td>
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<tr>
<td>Amplitude/Time Constant (mM/s)</td>
<td>0.47 ± 0.14</td>
<td>0.31 ± 0.09 *</td>
</tr>
<tr>
<td>[PCr] Slow Amplitude (mM)</td>
<td>3.0 ± 3.0</td>
<td>1.9 ± 2.3</td>
</tr>
<tr>
<td>End-Exercise [PCr] (mM)</td>
<td>14.2 ± 5.6</td>
<td>19.0 ± 8.0 *</td>
</tr>
<tr>
<td><strong>Recovery</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline [PCr] (mM)</td>
<td>14.2 ± 5.6</td>
<td>19.0 ± 8.0 *</td>
</tr>
<tr>
<td>Time Constant (s)</td>
<td>41 ± 11</td>
<td>44 ± 6</td>
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<tr>
<td>[PCr] Amplitude (mM)</td>
<td>20.2 ± 4.1</td>
<td>19.5 ± 4.6</td>
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<tr>
<td>Amplitude/Time Constant (mM/s)</td>
<td>0.50 ± 0.10</td>
<td>0.45 ± 0.18</td>
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<tr>
<td>[PCr] Slow Amplitude (mM)</td>
<td>0.7 ± 0.4</td>
<td>0.7 ± 1.0</td>
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<tr>
<td>End-Recovery [PCr] (mM)</td>
<td>35.0 ± 1.4</td>
<td>40.0 ± 3.7 *</td>
</tr>
</tbody>
</table>

* = P<0.05.
\[ \tau = 14 \text{ s} \]

\[ \tau = 15 \text{ s} \]

\[ \tau = 25 \text{ s} \]

\[ \tau = 27 \text{ s} \]

\[ \tau = 14 \text{ s} \]
A

$\tau = 72 \text{ s}$

$\tau = 54 \text{ s}$

B

$\tau = 44 \text{ s}$

$\tau = 41 \text{ s}$