The role of oxygen in determining phosphocreatine onset kinetics in exercising humans

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^{31}P-magnetic resonance spectroscopy was used to study phosphocreatine (PCr) onset kinetics in exercising human gastrocnemius muscle under varied fractions of inspired O_2 (F_{IO_2}). Five male subjects performed three identical work bouts (5 min duration; order randomised) at a submaximal workload while breathing 0.1, 0.21 or 1.0 F_{IO_2}. Either a single or double exponential model was fitted to the PCr kinetics. The phase I τ (0.1, 38.6 $\pm$ 7.5; 0.21, 34.5 $\pm$ 7.9; 1.0, 38.6 $\pm$ 9.2 s) and amplitude, A_1 (0.1, 0.34 $\pm$ 0.03; 0.21, 0.28 $\pm$ 0.05; 1.0, 0.28 $\pm$ 0.03,% fall in PCr) were invariant (both $P > 0.05$) across F_{IO_2} trials. The initial rate of change in PCr hydrolysis at exercise onset, calculated as A_1/τ_1 (%PCr reduction s^{-1}), was the same across F_{IO_2} trials. A PCr slow component (phase II) was present at an F_{IO_2} of 0.1 and 0.21; however, breathing 1.0 F_{IO_2} ablated the slow component. The onset of the slow component resulted in a greater ($P \leq 0.05$) overall percentage fall in PCr (both phase I and II) as F_{IO_2} decreased (0.43 $\pm$ 0.05, 0.34 $\pm$ 0.05, 0.28 $\pm$ 0.03) for 0.1, 0.21 and 1.0 F_{IO_2}, respectively. These data demonstrate that altering F_{IO_2} does not affect the initial phase I PCr onset kinetics, which supports the notion that O_2 driving pressure does not limit PCr kinetics at the onset of submaximal exercise. Thus, these data imply that the manner in which microvascular and intracellular P_{O_2} regulates PCr hydrolysis in exercising muscle is not due to the initial kinetic fall in PCr at exercise onset.

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Across the rest to work transition, [ATP] in working muscle is maintained by phosphocreatine (PCr) hydrolysis in a reaction catalysed by creatine kinase (CK). After a few seconds (if not immediately), both glycolysis (Connett *et al.* 1990; Howlett *et al.* 1999) and oxidative phosphorylation (Bangsbo, 2000; Behnke *et al.* 2003; Kindig *et al.* 2003) are activated. A direct proportionality between the kinetics of pulmonary $\dot{V}_{O_2}$ and PCr hydrolysis has been demonstrated previously (Mahler, 1985; Meyer, 1988), and thus the time course of pulmonary $\dot{V}_{O_2}$ onset kinetics has been related to changing [PCr] in muscle at the start of exercise (Marsh *et al.* 1993; Barstow *et al.* 1994; McCann *et al.* 1995; McCreary *et al.* 1996; Rossiter *et al.* 1999). In addition, the steady state level to which PCr falls is correlated positively to the steady state level of $\dot{V}_{O_2}$ during exercise (Mahler, 1985; Meyer, 1988). These data suggest that PCr hydrolysis (specifically the increase in inorganic phosphate) is a potent regulator of oxidative phosphorylation (Meyer, 1988), and that PCr hydrolysis and regulation of oxidative phosphorylation are tightly coupled (Meyer & Foley, 1996).

Our laboratory has demonstrated previously that the tight coupling between the steady state levels of PCr depletion and $\dot{V}_{O_2}$ can be dissociated by altering O_2 availability (specifically, microvascular and intracellular P_{O_2}; Richardson *et al.* 1999) during exercise by breathing varied fractions of inspired oxygen (F_{IO_2}) (Haseler *et al.* 1998). Additionally, pulmonary $\dot{V}_{O_2}$ kinetics have been modulated in an O_2-dependent manner by altering the F_{IO_2} (Hughes *et al.* 1968; Linnarsson *et al.* 1974; Engelen *et al.* 1996) although other investigations have reported invariant $\dot{V}_{O_2}$ kinetics in the face of altered F_{IO_2} (Linnarsson, 1974; Hughson & Kowalchuk, 1995). To date, whether PCr onset kinetics are O_2 dependent has not been tested. Given the tight relationship between pulmonary $\dot{V}_{O_2}$ and PCr hydrolysis, one possible scenario is that PCr kinetics will follow suit with $\dot{V}_{O_2}$ kinetics (Hughes *et al.* 1968; Linnarsson *et al.* 1974; Engelen *et al.*

This manuscript is dedicated to our friend and colleague Casey Kindig who tragically died after the submission of this manuscript for publication.

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1996) and be slowed as F_{IO_2} is reduced. Alternatively, despite the fact that $\dot{V}_{O_2}$ kinetics can be slowed with hypoxia due to the necessity for O_2 to serve as the terminal electron acceptor within the electron transport chain, PCr hydrolysis is catalysed by CK in a process largely dependent upon the metabolite signals arising from the contractile sites. Thus, regardless of O_2 availability, PCr kinetics may remain unchanged due to the similar ATP demand of work.

At low work intensities (moderate work domain), pulmonary $\dot{V}_{O_2}$ increases in a monoexponential fashion preceded by a short time delay. During work performed above the lactate threshold (i.e. heavy and severe exercise domains), a secondary rise in $\dot{V}_{O_2}$ occurs around 2–3 min after exercise onset (Whipp & Wasserman, 1972; Gaesser & Poole, 1996). This secondary rise in pulmonary $\dot{V}_{O_2}$, which has been termed the slow component, only occurs during a sustained lactic acidosis and represents an increased O_2 cost for a constant work rate. A recent study demonstrated a slow component of PCr onset kinetics with similar magnitude and time course to the $\dot{V}_{O_2}$ slow component (Rossiter *et al.* 2002). It has been well established that altering F_{IO_2} can alter the absolute work rate at which the lactate threshold occurs (Welch, 1982; Hogan *et al.* 1983). Thus it is possible, during work performed at an intensity above the lactate threshold, that hyperoxia may shift the work intensity from the heavy to the moderate domain. If true, in this scenario one might reasonably expect an ablation of the $\dot{V}_{O_2}$ slow component under hyperoxic conditions with a similar observation for the slow component of PCr onset kinetics.

To resolve these issues, we examined the effect of F_{IO_2} on PCr onset kinetics, measured by ^{31}P magnetic resonance spectroscopy (MRS), in human subjects performing plantar flexion exercise at a work intensity that elicited a 'slow component' during normoxia while breathing varied F_{IO_2} (0.1, 0.21 and 1.00).

Methods

Subjects

Five healthy men, aged 21–42 years, volunteered to participate in this study and gave written informed consent. The Human Subjects Committee of the University of California, San Diego, approved the study in accordance with the Declaration of Helsinki. Subjects were all active, ranging from recreational to well-trained athletes. The subjects refrained from strenuous activity for 24 h prior to data collection.

Exercise protocol

Subjects were familiarized with plantar flexion exercise in the confines of a whole body MRI system. At this time, a work rate which elicited a PCr slow component while breathing room air was determined which corresponded with ~60% of maximum work rate for each subject. Subjects performed constant load submaximal plantar flexion at this intensity (7.2 ± 0.6 W) at a frequency of 1 Hz (maintained with the aid of an electronic metronome) while lying supine in a superconducting 1.5 T magnet. At each F_{IO_2} (0.1, 0.21 and 1.00), subjects performed a 5 min warm-up followed by 5 min of rest, and then 5 min of exercise followed by 5 min of recovery. Subjects were allowed 30 min of rest between each complete exercise bout. MRS data were acquired continuously for 2 min before exercise, and for the 5 min exercise bout. The order of the three treatments was randomized and blinded to the subjects to minimize any putative ordering effects.

Arterial saturation and arterial P_{O_2}

Throughout each exercise bout, subjects breathed through a low resistance two-way breathing valve (2700; Hans-Rudolph Inc., Kansas City, MO, USA). Arterial O_2 saturation was monitored continuously throughout the experiment with a finger probe oximeter (Omni-Trak, In Vivo Research Inc., USA). During previous studies (Haseler *et al.* 1998; Haseler *et al.* 1999) a high correlation ($r^2 = 0.9$) between arterial O_2 saturation calculated from end-tidal O_2 gas measurement and that measured with this oximeter system was observed. These saturations were then used to calculate arterial P_{O_2} using the Hill equation, assuming a normal O_2 half-saturation pressure (P_{50}).

^{31}P MRS

MRS was performed using a clinical 1.5 T General Electric Signa system (5.4.2 version) operating at 25.86 MHz for ^{31}P. ^{31}P MRS data were acquired with a transmit/receive surface coil (diameters 20 and 10 cm, respectively) placed under the calf at its maximum diameter. The centring of the leg over the coil was confirmed by T1 weighted ^{1}H localizing images obtained in the axial plane and the coil was repositioned if the major part of the gastrocnemius muscle was not within this range. Shimming on the proton signal from tissue water optimized magnetic field homogeneity and the ^{31}P MRS signal was optimized by pre-scan transmitter gain adjustment so that ~72% of the signal acquired was from tissue within 5 cm of the surface coil. A 500 μs hard pulse was used for signal excitation. The

spectral width was 2500 Hz, and a single free induction decay (FID) was acquired every 4 s for 2 min prior to the onset of exercise and for the 5 min of exercise. As a result the data were expressed with a time resolution of 4 s.

Data analysis

Data were processed using SAGE/IDL software General Electric Medical Systems on a Silicon Graphics Indigo workstation. Each FID consisted of 1024 complex points and was processed with 5 Hz exponential line broadening prior to zero filling and Fourier transformation. All spectra were manually phased using zero and first order phase corrections. There were no phase variations between rest and exercise during the experiment. The levels of PCr determined from the intensity of that peak were normalized to 100% using the average value obtained from the last 40 s of rest acquired for each subject as a reference. Muscle intracellular pH was calculated from the chemical shift difference (δ) of the P_i peak relative to the PCr peak using the following equation:

$$\mathrm{pH} = 6.75 + \log[(\delta - 3.27)/(5.69 - \delta)]$$

(Taylor *et al.* 1983). Signal to noise ratios ($\sim$30 : 1) were sufficient to allow PCr levels to be determined with a temporal resolution of 4 s during the rest to exercise transition.

Kinetic analysis

Curve fitting (Kaleidagraph 3.5, Synergy software, Reading PA, USA) was performed on the PCr data using a monoexponential function:

$$\mathrm{PCr}(t) = \mathrm{PCr_b} - \Delta\mathrm{PCr_{ss}}(1 - \mathrm{e}^{-t/\tau_1})$$

and a double-exponential function

$$\mathrm{PCr}(t) = \mathrm{PCr_b} - \Delta\mathrm{PCr_{primary}}(1 - \mathrm{e}^{-t/\tau}) + \Delta\mathrm{PCr_{secondary}}(1 - \mathrm{e}^{-(t-\mathrm{TD})/\tau_2})$$

where $\mathrm{PCr}(t)$, $\mathrm{PCr_b}$ and $\Delta\mathrm{PCr_{ss}}$ are PCr at time t, baseline (pre-exercise) and the fall in PCr from resting baseline to steady-state exercising values, respectively. For the more complex double-exponential model, $\Delta\mathrm{PCr_{primary}}$ and $\Delta\mathrm{PCr_{secondary}}$ designate the asymptotic value to which that component of the ΔPCr is projecting. Figure 1 is a schematic diagram illustrating the kinetic analysis described above and referred to in the results. The τ_1 and τ_2 are the respective time constants and TD is the independent time delay prior to the onset of the secondary phase (slow component). Goodness of fit was assessed via visual inspection and analysis of the residual fit to the model as well as the coefficient of determination and Chi square values. In addition, time to 50% of the overall fall (T_{50}) was calculated, independent of modelling procedures.

Statistical analysis

Data were tested with repeated measures ANOVA (Tukey's *post hoc* test) using a commercially available software package (InStat, GraphPad Software Inc., San Diego, CA, USA). Data were considered significantly different when $P \leq 0.05$. Data are presented as means ± S.E.M.

Results

Normalized PCr data at rest and across the three exercise bouts for a representative subject (raw data (4 s time resolution) with model fit for each trial) are shown in Fig. 2. The PCr phase I amplitude (A_1), expressed as a fraction of resting PCr levels, was not different (0.1, 0.34 ± 0.03; 0.21, 0.28 ± 0.05; 1.0, 0.28 ± 0.03; $P > 0.05$) across the range of F_{IO_2} values employed in this study (Fig. 3). A PCr slow component was present at an F_{IO_2} of 0.1 and 0.21; however, at 1.0 F_{IO_2} there was no discernible slow component (Figs 2 and 3). This was confirmed statistically with significantly greater ($P < 0.05$) coefficient of determination and Chi square values for the 2- *versus* 1-component model at F_{IO_2} of 0.1 and 0.21, yet unchanged (both $P > 0.05$) values at $F_{IO_2} = 1.0$. The onset of the slow component (i.e. TD) at 0.1 and 0.21 F_{IO_2} occurred 181 ± 16 and 188 ± 27 s into the exercise bout, respectively. The overall percentage fall in PCr (including phase II 'slow component') increased

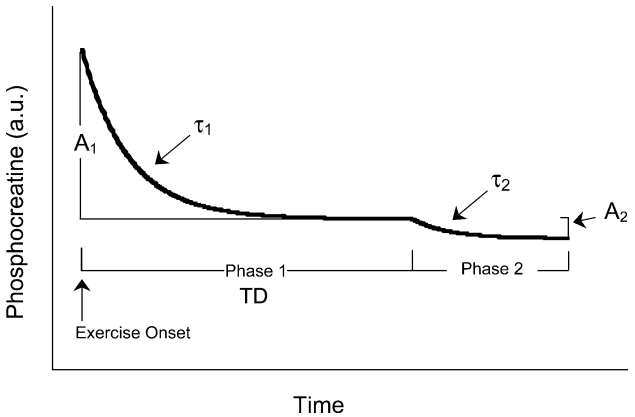

Figure 1. A schematic diagram of the model and kinetic parameters used to fit the data as described in Methods and Results

A_1, amplitude of the initial phase I fall in PCr; τ_1, time constant for the phase I fall in PCr; A_2, amplitude of the phase II fall in PCr (slow component); τ_2, time constant of the phase II kinetics; TD, time delay prior to slow component onset.

significantly as F_{IO_2} decreased (0.1, 0.43 ± 0.05; 0.21, 0.34 ± 0.05; 1.0, 0.28 ± 0.03; Fig. 3).

Figure 4 demonstrates that the PCr A_1 time constants (i.e. τ_1) were invariant ($P > 0.05$) across the varied F_{IO_2} trials (0.1, 38.6 ± 7.5; 0.21, 34.5 ± 7.9; 1.0 38.6 ± 9.2 s). In addition, the initial rate of change in PCr hydrolysis at exercise onset, calculated as A_1/τ_1 (%PCr reduction s^{-1}), was the same across F_{IO_2} trials (Table 1). However, if the 'speed of the PCr fall at exercise onset' is simply assessed as time to 50% of the overall PCr fall (i.e. independent of modelling procedures), the T_{50} was significantly slowed as F_{IO_2} was reduced from 1.0 to 0.1 (Table 1).

Intracellular pH, calculated from the ^{31}P MRS spectra obtained during the last 40 s of exercise, was significantly greater during the hyperoxic condition and lower during the hypoxic condition, than during normoxic breathing (Table 1). The arterial O_2 saturations reported in Table 1 were significantly reduced with hypoxic breathing compared to normoxia with only a minimal increase with hyperoxia. The arterial P_{O_2} calculated from the arterial saturations increased with increasing F_{IO_2} (Table 1).

Discussion

To our knowledge, this is the first study to investigate the effect of F_{IO_2} on PCr onset kinetics in humans performing dynamic submaximal plantar flexion exercise. In the present study, we altered F_{IO_2} in order to evoke large changes in arterial P_{O_2}. Thus, reducing F_{IO_2} from 1.0 to 0.21–0.1 resulted in a reduced microvascular P_{O_2} and ultimately less diffusive O_2 flux to the myocyte at a given workload regardless of O_2 delivery (Richardson *et al.* 1999). Two key and novel findings in this current study are that (1) altered F_{IO_2} did not alter phase I PCr on-kinetics and (2) a hyperoxic F_{IO_2} ablated the PCr slow component manifest during normoxic and hypoxic exercise. The data demonstrate that the initial phase of the fall in PCr does not depend critically upon the O_2 driving gradient from the microvasculature to the mitochondria, at least across the range evoked by breathing gases across the F_{IO_2} range from 0.1 to 1.0. The ablation of the PCr slow component with an F_{IO_2} of 1.0 suggests that P_{O_2} (both microvascular and intracellular) is important in the regulation of PCr hydrolysis after the initial phase I kinetics. As a whole, these data suggest that the mechanism by which altering F_{IO_2} impacts upon the cellular metabolic state in exercising muscle is not related to the speed of the initial PCr fall, but rather to events occurring thereafter.

$\dot{V}_{O_2}$ and PCr hydrolysis

Recently, Rossiter *et al.* (2002) simultaneously acquired both muscle PCr kinetics and pulmonary $\dot{V}_{O_2}$ kinetics across the rest to work transition in humans performing both moderate and heavy intensity knee extensor exercise. Cogent to the current investigation, two key findings were observed. First, the time course of the fall in PCr matched that for the rise in $\dot{V}_{O_2}$ at exercise onset as

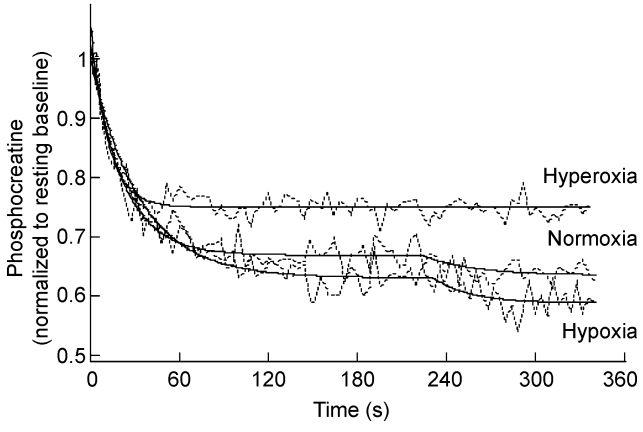

Figure 2. Raw data and the model fit for PCr hydrolysis at exercise onset in a representative subject under each F_{IO_2} (hypoxia 0.1, normoxia 0.21, hyperoxia 1.0)
Note that the slow component of PCr hydrolysis, manifest in the hypoxic and normoxic trials, is ablated in the hyperoxic condition.

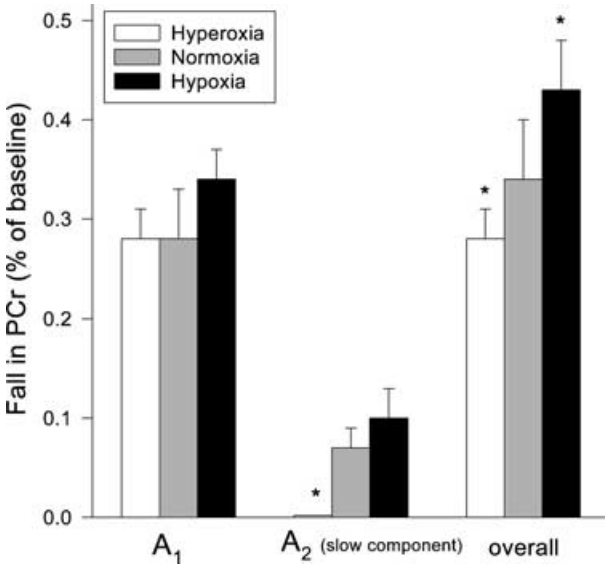

Figure 3. The effect of F_{IO_2} on the phase I, phase II and overall PCr amplitudes
The amplitude of the initial phase I fall of PCr (A_1), expressed as a fraction of resting PCr, was not different as F_{IO_2} was increased. A slow component of PCr was present at F_{IO_2} of 0.1 and 0.21 (A_2). The overall fall in PCr, both A_1 and A_2, increased significantly as F_{IO_2} was decreased.

previously described (Barstow *et al.* 1994; McCreary *et al.* 1996). Second, the onset of the $\dot{V}_{O_2}$ slow component was in close temporal proximity with a slow component of PCr hydrolysis. These data support the tenet that the slow component originates within the exercising muscle (Poole, 1994; Gaesser & Poole, 1996). Also, these findings set the stage to discuss the mechanisms for the PCr slow component acquired in the current study in the context of data reported previously for pulmonary $\dot{V}_{O_2}$.

Effect of F_{IO_2} on metabolism

It has long been accepted that altering F_{IO_2} can result in a change in the absolute work rate at which the lactate threshold is reached during a ramped increase in work. Welch (1982) demonstrated that, during cycle ergometry, lowering the F_{IO_2} shifted the lactate threshold to the left (i.e. a lower absolute work rate and $\dot{V}_{O_2}$%) whereas breathing hyperoxic gas shifted the lactate threshold to the right (Welch, 1982). The interpretation of these findings are confounded by the likelihood that blood flow to the working muscle was elevated during hypoxia and possibly reduced during hyperoxia to counteract the reduced haemoglobin saturation such that oxygen delivery ($\dot{Q}_{O_2}$) remained unchanged (Rowell *et al.* 1986; Hogan *et al.* 1988). However, microvascular P_{O_2} would be elevated at each progressively greater F_{IO_2}, which would increase the potential for O_2 flux from the capillary to the mitochondrion during the exercise bout.

At the transition to moderate intensity exercise, the speed of the pulmonary $\dot{V}_{O_2}$ response does not depend critically on O_2 availability in normal healthy subjects whereas above the lactate threshold, the $\dot{V}_{O_2}$ kinetics may be dependent, in part, on the O_2 concentration (Hughson & Morrissey, 1982; Gerbino *et al.* 1996; Tschakovsky & Hughson, 1999). To date, the specific P_{O_2} within the microcirculation that may alter the initial metabolic response from rest to high-intensity exercise is not known. Wilson & Rumsey (1988) demonstrated that a P_{O_2} of 30 Torr requires compensatory changes in the phosphorylation and redox potential to maintain mitochondrial respiration in cell suspensions but the changes are minor until the P_{O_2} drops to around 10 Torr. This finding has been corroborated during small muscle mass exercise in humans, in which manipulations in F_{IO_2} induced significant alterations in steady-state PCr levels and intracellular P_{O_2} (Haseler *et al.* 1998; Richardson *et al.* 1999).

Furthermore, and possibly more pertinent to the current study, previous investigations have demonstrated that alterations in F_{IO_2} may alter the kinetics of $\dot{V}_{O_2}$ in an O_2-dependent manner (Linnarsson *et al.* 1974; Engelen *et al.* 1996; MacDonald *et al.* 1997). However, other investigations have observed no changes in $\dot{V}_{O_2}$ kinetics with altered F_{IO_2} (Linnarsson, 1974; Hughson & Kowalchuk, 1995). In a series of investigations by Grassi and colleagues using the isolated dog hindlimb preparation, acute manipulations in $\dot{Q}_{O_2}$ and/or availability of O_2 did not affect $\dot{V}_{O_2}$ on-kinetics during moderate intensity exercise (Grassi *et al.* 1998*a*,*b*), but increasing O_2 delivery did speed $\dot{V}_{O_2}$ kinetics in response to maximal intensity exercise (Grassi *et al.* 2000). The data presented here suggest that altering the F_{IO_2} does not affect the primary amplitude of PCr kinetics in humans, at least during small muscle mass exercise, which implies that the metabolic perturbation seen with reduced O_2 availability during steady state exercise is not due to altered primary (initial) PCr kinetics (Haseler *et al.* 1998).

Kinetics of initial PCr fall

At the onset of exercise, the rate of PCr hydrolysis increases to meet the increase in ATP demand. PCr hydrolysis and oxidative phosphorylation are tightly coupled such that PCr falls with the same time course and in proportion to pulmonary $\dot{V}_{O_2}$ (Mahler, 1985; Rossiter *et al.* 1999). The regulation of this kinetic response has been the subject of some controversy with investigations focusing on two

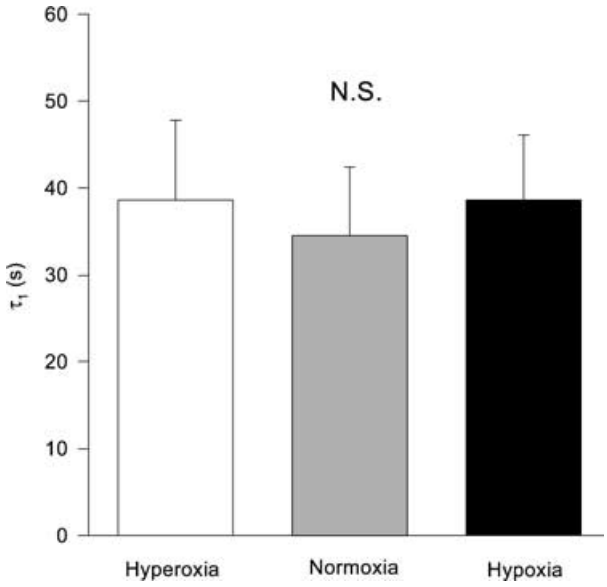

Figure 4. The time constants, τ_1, of the initial phase I kinetics of PCr hydrolysis
The initial phase I kinetics of PCr breakdown at the onset of exercise were invariant ($P > 0.05$) across the varied F_{IO_2} trials.

Table 1. A summary of data acquired under each F_{IO_2} condition. The intracellular pH was determined for the last 40 s of exercise

	Hyperoxia	Normoxia	Hypoxia
T_{50} (s)	16.6 ± 3.7*	26.6 ± 4.9	38.6 ± 8.0*
A_1/τ_1 (s)	0.01 ± 0.003	0.01 ± 0.003	0.01 ± 0.002
Intracellular pH	7.09 ± 0.01*	7.07 ± 0.01	7.01 ± 0.02*
S_{aO_2}	100 ± 1.0	98.2 ± 0.3	70.1 ± 3.6*
Arterial P_{O_2}	650 ± 7*	120 ± 4	42 ± 10*

Significantly different from Normoxia, $P < 0.05$.

hypotheses: (1) the oxidative enzyme inertia hypothesis (Cerretelli *et al.* 1980; Grassi *et al.* 1998*a*; Grassi *et al.* 1998*b*), where the delayed rise in $\dot{V}_{O_2}$ reflects a metabolic inertia determined solely by levels of cellular metabolic controllers (phosphorylation potential and redox state) and/or mitochondrial enzyme activation, and (2) the O_2 delivery hypothesis, where the speed of the rise in $\dot{V}_{O_2}$ is related to the rate of adjustment of O_2 delivery to the exercising muscles (Gerbino *et al.* 1996; Hughson *et al.* 1996; MacDonald *et al.* 1997). A complex interaction between the cellular metabolic state, enzyme activation state, and O_2 availability is likely to be responsible for determining the rate of change in oxidative phosphorylation at the onset of exercise (Tschakovsky & Hughson, 1999).

In the current investigation, O_2 availability was manipulated by having the subjects exercise while breathing a range of F_{IO_2}. As discussed in the previous section, the effect of varying F_{IO_2} on convective O_2 delivery is dampened by an alteration in muscle blood flow (Rowell *et al.* 1986). The most significant effect of hyperoxia is to raise blood P_{O_2}, as Hb saturation is already close to its ceiling in normoxia (and muscle blood flow tends to fall in hyperoxia). Hence, it is suggested that in hyperoxia the driving gradient from blood to muscle was enhanced resulting in an elevated intracellular P_{O_2}; conversely, hypoxia may result in elevated blood flow, but a reduced O_2 driving gradient (Richardson *et al.* 1999). The diffusive component of O_2 transport would be enhanced with increasing F_{IO_2} due to the increasing microvascular and intracellular P_{O_2}. The time constant of the initial fall in PCr was unchanged with increasing F_{IO_2} (Fig. 4), as was the initial rate of change in PCr hydrolysis (Table 1) consistent with data reported by Kindig *et al.* (2003) in frog single skeletal muscle fibres. The present data suggest that O_2 availability (diffusive component with altered F_{IO_2}) is not a key determinant of the initial PCr hydrolysis response at the onset of exercise in humans. However, what this investigation fails to address is whether $\dot{V}_{O_2}$ kinetics were altered during the plantar flexion exercise with changing F_{IO_2}. One postulate would be that the $\dot{V}_{O_2}$ kinetics would not be changed in the face of invariant PCr kinetics and thus the PCr and $\dot{V}_{O_2}$ kinetic relationship is maintained as previously described (Mahler, 1985; Rossiter *et al.* 1999). However, while oxidative phosphorylation is ultimately dependent upon O_2 as the terminal electron acceptor within the electron transport chain, the CK-catalysed breakdown of PCr is primarily dependent upon the metabolic signals arising from the contractile sites. Thus, the 0.1 F_{IO_2} condition represents a unique situation in which PCr kinetics may be dissociated from $\dot{V}_{O_2}$ kinetics.

PCr hydrolysis and the slow component

It has been well documented that a $\dot{V}_{O_2}$ slow component occurs solely during work performed above the lactate threshold (Gaesser & Poole, 1996). This apparent reduction in muscle 'efficiency' does not occur immediately, but rather some 2–3 min after the onset of aerobic work. This additional $\dot{V}_{O_2}$ gain slows the overall speed of the $\dot{V}_{O_2}$ kinetic response (Whipp & Wasserman, 1972; Linnarsson, 1974). Mechanisms responsible for the additional O_2 cost associated with the $\dot{V}_{O_2}$ slow component have yet to be resolved (Gaesser & Poole, 1996), although recent research suggests that it may be related to muscle fibre or muscle fibre type recruitment consequent to the sustained lactic acidemia (Shinohara & Moritani, 1992; Poole, 1994; Ozyener *et al.* 2001; Burnley *et al.* 2002).

Previous investigations have been able to manipulate the slow component to a certain extent. Originally, it was demonstrated that exercise training shifts the lactate threshold to an overall greater absolute workload, such that performing work, which would have evoked a slow component prior to training, will no longer do so (Gaesser & Poole, 1996). Furthermore, Kindig *et al.* (2001) demonstrated an earlier onset of the slow component, yet unaltered amplitude, after nitric oxide synthase inhibition, which was thought to be due to an early onset of fatigue induced by reduced O_2 delivery. Burnley *et al.* (2000) have observed a significantly reduced slow component amplitude following an initial 'priming' bout of exercise. Additionally, one investigation by MacDonald *et al.* (1997) demonstrated that hyperoxia ($F_{IO_2} = 0.70$) shortened the $\dot{V}_{O_2}$ mean response time and reduced the amplitude of the slow component. One shortcoming of that investigation was the inability to utilize 100% O_2 (i.e. the inability to perform the Haldane transformation) and thereby further increase the O_2 driving gradient

from the microvasculature to the myocyte. In the current investigation, an F_{IO_2} of 1.00 was employed and this manipulation ablated the slow component of PCr hydrolysis. Given that the slow component is only evidenced at work performed above the lactate threshold, the most logical explanation for this would be that hyperoxia shifted the lactate threshold such that during hyperoxic breathing, the work intensity was actually in the moderate rather than the heavy domain. Additionally, and along a similar line to that of MacDonald *et al.* (1997), the greater O_2 driving gradient may have reduced the fatigue incurred, thus slowing the recruitment of additional fibres thought to be integral to the mechanism of the slow component.

Summary

These data demonstrate that altering F_{IO_2} does not alter the initial phase I PCr onset kinetics, which supports the notion that O_2 driving pressure does not limit energetics at the onset of submaximal exercise. However, the ablation of the slow component with hyperoxic breathing suggests an important role for microvascular and intracellular P_{O_2} in the regulation of PCr hydrolysis in exercising muscle after the initial phase I of PCr kinetics which modulates the level of metabolic perturbation induced by varying F_{IO_2}.

References

Bangsbo J (2000). Muscle oxygen uptake in humans at onset of and during intense exercise. *Acta Physiol Scand* **168**, 457–464.

Barstow TJ, Buchthal S, Zanconato S & Cooper DM (1994). Muscle energetics and pulmonary oxygen uptake kinetics during moderate exercise. *J Appl Physiol* **77**, 1742–1749.

Behnke BJ, McDonough P, Padilla DJ, Musch TI & Poole DC (2003). Oxygen exchange profile in rat muscles of contrasting fibre types. *J Physiol* **549**, 597–605.

Burnley M, Doust JH, Ball D & Jones AM (2002). Effects of prior heavy exercise on VO_2 kinetics during heavy exercise are related to changes in muscle activity. *J Appl Physiol* **93**, 167–174.

Burnley M, Jones AM, Carter H & Doust JH (2000). Effects of prior heavy exercise on phase II pulmonary oxygen uptake kinetics during heavy exercise. *J Appl Physiol* **89**, 1387–1396.

Cerretelli P, Rennie DW & Pendergast DR (1980). Kinetics of metabolic transients during exercise. In *Exercise Bioenergetics and Gas Exchange*, ed. Cerrretelli P & Whipp BJ, pp. 187–209. Elsevier, Amsterdam.

Connett RJ, Honig CR, Gayeski TEJ & Brooks GA (1990). Defining hypoxia: a systems view of VO_2, glycolysis, energetics, and intracellular PO_2. *J Appl Physiol* **68**, 833–842.

Engelen M, Porszasz J, Riley M, Wasserman K, Maehara K & Barstow TJ (1996). Effects of hypoxic hypoxia on O_2 uptake and heart rate kinetics during heavy exercise. *J Appl Physiol* **81**, 2500–2508.

Gaesser GA & Poole DC (1996). The slow component of oxygen uptake kinetics in humans. *Exerc Sport Sci Rev* **24**, 35–71.

Gerbino A, Ward SA & Whipp BJ (1996). Effects of prior exercise on pulmonary gas-exchange kinetics during high-intensity exercise in humans. *J Appl Physiol* **80**, 99–107.

Grassi B, Gladden LB, Samaja M, Stary CM & Hogan MC (1998*a*). Faster adjustment of O_2 delivery does not affect V_{O2} on-kinetics in isolated in situ canine muscle. *J Appl Physiol* **85**, 1394–1403.

Grassi B, Gladden LB, Stary CM, Wagner PD & Hogan MC (1998*b*). Peripheral O_2 diffusion does not affect V_{O2} on-kinetics in isolated insitu canine muscle. *J Appl Physiol* **85**, 1404–1412.

Grassi B, Hogan MC, Kelley KM, Aschenbach WG, Hamann JJ, Evans RK, Patillo RE & Gladden LB (2000). Role of convective O_2 delivery in determining VO_2 on-kinetics in canine muscle contracting at peak VO_2. *J Appl Physiol* **89**, 1293–1301.

Haseler LJ, Hogan MC & Richardson RS (1999). Skeletal muscle phosphocreatine recovery in exercise trained humans is dependent on O_2 availability. *J Appl Physiol* **86**, 2013–2018.

Haseler LJ, Richardson RS, Videen JS & Hogan MC (1998). Phosphocreatine hydrolysis during submaximal exercise: the effect of FIO_2. *J Appl Physiol* **85**, 1457–1463.

Hogan MC, Cox RH & Welch HG (1983). Lactate accumulation during incremental exercise with varied inspired oxygen fractions. *J Appl Physiol* **55**, 1134–1140.

Hogan MC, Roca J, Wagner PD & West JB (1988). Limitation of maximal O_2 uptake and performance by acute hypoxia in dog skeletal muscle in situ. *J Appl Physiol* **65**, 815–821.

Howlett RA, Heigenhauser GJ, Hultman E, Hollidge-Horvat MG & Spriet LL (1999). Effects of dichloroacetate infusion on human skeletal muscle metabolism at the onset of exercise. *Am J Physiol* **277**, E18–E25.

Hughes RL, Clode M, Edwards RHT, Goodwin TJ & Jones NL (1968). Effect of inspired O_2 on cardiopulmonary and metabolic responses to exercise in man. *J Appl Physiol* **24**, 336–347.

Hughson RL & Kowalchuk JM (1995). Kinetics of oxygen uptake for submaximal exercise in hyperoxia, normoxia, and hypoxia. *Can J Appl Physiol* **20**, 198–210.

Hughson RL & Morrissey M (1982). Delayed kinetics of respiratory gas exchange in the transition from prior exercise. *J Appl Physiol* **52**, 921–929.

Hughson RL, Shoemaker JK, Tschakovsky ME & Kowalchuk JM (1996). Dependence of muscle VO_2 on blood flow dynamics at onset of forearm exercise. *J Appl Physiol* **81**, 1619–1626.

Kindig CA, Howlett RA & Hogan MC (2003). Effect of extracellular PO_2 on the fall in intracellular PO_2 in contracting single myocytes. *J Appl Physiol* **94**, 1964–1970.

Kindig CA, McDonough P, Erickson HH & Poole DC (2001). Effect of L-NAME on oxygen uptake kinetics during heavy-intensity exercise in the horse. *J Appl Physiol* **91**, 891–896.

Linnarsson D (1974). Dynamics of pulmonary gas exchange and heart rate changes at start and end of exercise. *Acta Physiol Scand Suppl* **415**, 1–68.

Linnarsson D, Karlsson J, Fagraeus L & Saltin B (1974). Muscle metabolites and oxygen deficit with exercise in hypoxia and hyperoxia. *J Appl Physiol* **36**, 399–402.

MacDonald M, Pedersen PK & Hughson RL (1997). Acceleration of VO_2 kinetics in heavy submaximal exercise by hyperoxia and prior high-intensity exercise. *J Appl Physiol* **83**, 1318–1325.

Mahler M (1985). First-order kinetics of muscle oxygen consumption, and an equivalent proportionality between QO_2 and phosphorylcreatine level. *J Gen Physiol* **86**, 135–165.

Marsh GD, Paterson DH, Potwarka JJ & Thompson RT (1993). Transient changes in muscle high-energy phosphates during moderate exercise. *J Appl Physiol* **75**, 648–656.

McCann DJ, Mole PA & Caton JR (1995). Phosphocreatine kinetics in humans during exercise and recovery. *Med Sports Exerc* **27**, 378–387.

McCreary CR, Chilibeck PD, Marsh GD, Paterson DH, Cunningham DA & Thompson RT (1996). Kinetics of pulmonary oxygen uptake and muscle phosphates during moderate intensity calf exercise. *J Appl Physiol* **81**, 1331–1338.

Meyer RA (1988). A linear model of muscle respiration explains monoexponential phosphocreatine changes. *Am J Physiol* **254**, C548–C553.

Meyer RA & Foley JM (1996). Cellular processes integrating the metabolic response to exercise. In *Handbook of Physiology*, ed. Rowell LB & Shepherd JT, pp. 841–869. American Physiological Society, Bethesda.

Ozyener F, Rossiter HB, Ward SA & Whipp BJ (2001). Influence of exercise intensity on the on- and off-transient kinetics of pulmonary oxygen uptake in humans. *J Physiol* **533**, 891–902.

Poole DC (1994). Role of exercising muscle in slow component of VO_2. *Med Sci Sport Exerc* **26**, 1335–1340.

Richardson RS, Leigh JS, Wagner PD & Noyszewski EA (1999). Cellular PO_2 as a determinant of maximal mitochondrial O_2 consumption in trained human skeletal muscle. *J Appl Physiol* **87**, 325–331.

Rossiter HB, Ward SA, Doyle VL, Howe FA, Griffiths JR & Whipp BJ (1999). Inferences from pulmonary O_2 uptake with respect to intramuscular [phosphocreatine] kinetics during moderate exercise in humans. *J Physiol* **518**, 921–932.

Rossiter HB, Ward SA, Howe FA, Kowalchuk JM, Griffiths JR & Whipp BJ (2002). Dynamics of intramuscular ^{31}P-MRS P_i peak splitting and the slow components of PCr and O_2 uptake during exercise. *J Appl Physiol* **93**, 2059–2069.

Rowell LB, Saltin B, Kiens B & Christensen NJ (1986). Is peak quadriceps blood flow in humans even higher during exercise with hypopoxemia? *Am J Physiol* **251**, H1038–H1044.

Shinohara M & Moritani T (1992). Increase in neuromuscular activity and oxygen uptake during heavy exercise. *Ann Physiol Anthropol* **11**, 257–262.

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

Tschakovsky ME & Hughson RL (1999). Interaction of factors determining oxygen uptake at the onset of exercise. *J Appl Physiol* **86**, 1101–1113.

Welch HG (1982). Hyperoxia and human performance: a brief review. *Med Sci Sport Exerc* **14**, 253–262.

Whipp BJ & Wasserman K (1972). Oxygen uptake kinetics for various intensities of constant load work. *J Appl Physiol* **33**, 351–356.

Wilson DF & Rumsey WL (1988). Factors modulating the oxygen dependence of mitochondrial oxidative phosphorylation. *Adv Exp Med Biol* **222**, 121–131.

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