

## Inferences from pulmonary O<sub>2</sub> uptake with respect to intramuscular [phosphocreatine] kinetics during moderate exercise in humans

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1. In the non-steady state of moderate intensity exercise, pulmonary O<sub>2</sub> uptake ( $\dot{V}_{p,O_2}$ ) is temporally dissociated from muscle O<sub>2</sub> consumption ( $\dot{V}_{m,O_2}$ ) due to the influence of the intervening venous blood volume and the contribution of body O<sub>2</sub> stores to ATP synthesis. A monoexponential model of  $\dot{V}_{p,O_2}$  without a delay term, therefore, implies an obligatory slowing of  $\dot{V}_{p,O_2}$  kinetics in comparison to  $\dot{V}_{m,O_2}$ .
2. During moderate exercise, an association of  $\dot{V}_{m,O_2}$  and [phosphocreatine] ([PCr]) kinetics is a necessary consequence of the control of muscular oxidative phosphorylation mediated by some function of [PCr]. It has also been suggested that the kinetics of  $\dot{V}_{p,O_2}$  will be expressed with a time constant within 10% of that of  $\dot{V}_{m,O_2}$ .
3.  $\dot{V}_{p,O_2}$  and intramuscular [PCr] kinetics were investigated simultaneously during moderate exercise of a large muscle mass in a whole-body NMR spectrometer. Six healthy males performed prone constant-load quadriceps exercise. A transmit–receive coil under the right quadriceps allowed determination of intramuscular [PCr];  $\dot{V}_{p,O_2}$  was measured breath-by-breath, in concert with [PCr], using a turbine and a mass spectrometer system.
4. Intramuscular [PCr] decreased monoexponentially with no delay in response to exercise. The mean of the time constants ( $\tau_{PCr}$ ) was 35 s (range, 20–64 s) for the six subjects.
5. Two temporal phases were evident in the  $\dot{V}_{p,O_2}$  response. When the entire  $\dot{V}_{p,O_2}$  response was modelled to be exponential with no delay, its time constant ( $\tau'_{\dot{V}_{p,O_2}}$ ) was longer in all subjects (group mean = 62 s; range, 52–92 s) than that of [PCr], reflecting the energy contribution of the O<sub>2</sub> stores.
6. Restricting the  $\dot{V}_{p,O_2}$  model fit to phase II resulted in matching kinetics for  $\dot{V}_{p,O_2}$  (group mean  $\tau_{\dot{V}_{p,O_2}} = 36$  s; range, 20–68 s) and [PCr], for all subjects.
7. We conclude that during moderate intensity exercise the phase II  $\tau_{\dot{V}_{p,O_2}}$  provides a good estimate of  $\tau_{PCr}$  and by implication that of  $\dot{V}_{m,O_2}$  ( $\tau_{\dot{V}_{m,O_2}}$ ).

The steady-state aerobic cost of a given work rate in humans is not appreciably different among subjects differing in age, gender, physical fitness or state of training (Hagberg *et al.* 1980; Babcock *et al.* 1994). Consequently, the clues to the control of muscular oxygen consumption ( $\dot{V}_{m,O_2}$ ), and pulmonary oxygen uptake ( $\dot{V}_{p,O_2}$ ), reside in their non-steady-state response profiles with respect to potential intramuscular controllers (Kushmerick *et al.* 1992). During the kinetic phase of a rest–moderate–work transition, [ATP] is maintained by phosphocreatine (PCr) breakdown via the creatine kinase reaction and also by oxidative

phosphorylation. While it is generally agreed that  $\dot{V}_{m,O_2}$  is controlled by features of this high energy phosphate bond splitting, the precise mechanisms by which the stressed muscle avoids what Chance *et al.* (1985) have termed ‘metabolic catastrophe’ remain to be elucidated.

The number of alternative mechanisms proposed for this control is great, and includes: control by the concentration of ADP (or inorganic phosphate; P<sub>i</sub>) through Michaelis–Menten enzyme kinetics (Chance & Williams, 1956) or via a ‘higher order’ model (Jeneson *et al.* 1996), and thermodynamic control through the ‘phosphorylation potential’

([ATP]/[ADP][P<sub>i</sub>]) (see Brown, 1992) and the changes in Gibbs free energy of cytosolic ATP hydrolysis ( $\Delta G_{\text{ATP}}$ ) (Kushmerick, 1983; Meyer, 1989; for a review see also Meyer & Foley, 1996). A small number of investigators have performed experiments to determine the dynamic profiles of both PCr and  $\dot{V}_{\text{p},\text{O}_2}$  in order to gain insight into these control mechanisms in humans. Barstow *et al.* (1994*a, b*) reported similar time constants for a fall in [PCr] and an increase in  $\dot{V}_{\text{p},\text{O}_2}$  during the non-steady state of muscular exercise. However, due to methodological constraints, these authors utilized different muscle groups operating over a different range of metabolic rates to obtain these results: plantar flexion to determine the time constant for [PCr] ( $\tau_{\text{PCr}}$ ), and cycle ergometry during a different session to determine the time constant for  $\dot{V}_{\text{p},\text{O}_2}$  ( $\tau_{\dot{V}_{\text{p},\text{O}_2}}$ ). Other investigators (McCreary *et al.* 1996) used identical modes of exercise when they compared  $\tau_{\text{PCr}}$ , measured by <sup>31</sup>P-magnetic resonance spectroscopy (<sup>31</sup>P-MRS) from the muscle (m.) lateralis gastrocnemius and m. soleus during plantar flexion, with  $\tau_{\dot{V}_{\text{p},\text{O}_2}}$ , which was, again, performed outside the magnet. However, the confidence of parameter estimation of  $\tau_{\dot{V}_{\text{p},\text{O}_2}}$  is highly dependent on the steady-state increment attained during the exercise (Lamarra *et al.* 1987). Plantar flexion, in the study of McCreary *et al.* (1996), produced only an  $\sim 100 \text{ ml min}^{-1}$  increment in  $\dot{V}_{\text{p},\text{O}_2}$ . The confidence limits of the parameter estimation with such small increments in  $\dot{V}_{\text{p},\text{O}_2}$  ( $\Delta \dot{V}_{\text{p},\text{O}_2, \text{ss}}$ ) are consequently poor, making differences in  $\tau$  values difficult to discern with adequate confidence. Therefore, while both of these studies imply that  $\tau_{\text{PCr}}$  and  $\tau_{\dot{V}_{\text{p},\text{O}_2}}$  are equal, further study of the issue is warranted.

The present study, therefore, was designed to compare the kinetics of [PCr] and  $\dot{V}_{\text{p},\text{O}_2}$  measured simultaneously during moderate intensity m. quadriceps exercise in a whole-body NMR magnet, with special reference to different strategies for discerning  $\tau_{\dot{V}_{\text{p},\text{O}_2}}$ .

## METHODS

Six healthy male volunteers aged between 21 and 59 years provided informed consent (as approved by the Institutional Ethics Committee for Human Experimentation) to participate in the study, and were cleared to exercise inside the bore of the MR scanner. All subjects were healthy and physically active but were not in competitive training, apart from one subject (subject 1) who competed at University level sport.

Each subject performed a series of habituation tests in the Laboratory of Human Physiology before progressing to the Magnetic Resonance Unit for data collection. During the habituation studies, work rates were determined for each subject that were moderate in intensity (i.e. a work rate that was below the estimated lactate threshold ( $\theta_L$ )). This was determined both from the profile of oxygen uptake at constant work rate (Whipp, 1996), and by the V-slope method (Beaver *et al.* 1986), defined as the slope change in pulmonary carbon dioxide output ( $\dot{V}_{\text{p},\text{CO}_2}$ ) relative to  $\dot{V}_{\text{p},\text{O}_2}$ . The work rate for each subject was chosen to produce the largest possible subthreshold increment in  $\dot{V}_{\text{p},\text{O}_2}$ .

The subjects lay prone inside the bore of the MR scanner with their feet suspended in rubber stirrups that formed an integral part of a

custom-designed plastic insert into the magnet bore (Fig. 1). The rubber stirrups provided a resistance against which the subject could perform rhythmic knee extension exercise of constant amplitude (i.e. the height extent of the magnet bore, approximately 30 cm) and constant frequency (32 pulses  $\text{min}^{-1}$ ) (Whipp *et al.* 1999). The subjects received an audible cue at 32 pulses  $\text{min}^{-1}$ , such that the contraction of the quadriceps of the non-dominant leg occurred in unison with the interrogation of the quadriceps of the dominant leg by magnetic resonance, i.e. the muscles that are sampled are stationary during the sampling and contracting between each sample acquisition. <sup>31</sup>P-MRS free induction decay (FID) signals were obtained every 1875 ms throughout the entire 16 min square wave exercise test protocol. Data were averaged over eight acquisitions (a <sup>31</sup>P spectrum every 15 s), which was the optimal compromise between spectral signal-to-noise and temporal resolution. Pulmonary oxygen uptake was determined breath-by-breath using algorithms described by Beaver *et al.* (1981).

The subject was 'strapped down' to the NMR scanner table by means of a non-distensible strap placed over the hips in order to minimize any extraneous movement (Fig. 1). Each subject performed a square wave exercise protocol: 240 s at rest, 360 s of constant-load exercise, and 360 s recovery. In order to increase the confidence of parameter estimation (see below), each subject repeated the exercise test twice with at least 6 min recovery between each subsequent test. One subject repeated the test three times. The work rate increments varied between approximately 30 and 80 W depending on the limits of moderate work for each subject. Work rate approximations were determined as the product of the amplitude of deflection of the exercising limbs, the frequency of deflection and the elastic coefficient of the rubber stirrups, which had been previously calculated over this length of deflection.

### Measurement of breath-by-breath gas exchange

Pulmonary gas exchange was measured using a computer and the algorithms of Beaver *et al.* (1981) (CaSE, Gillingham, UK; Whipp *et al.* 1999). Expired volume was measured by a volume measuring module (VMM) with non-magnetic custom-built turbine with stainless-steel mountings and extended cable (Interface Associates, Laguna Nigel, CA, USA), and calibrated with a 3 l syringe (Hans Rudolph, Kansas City, MO, USA). Respired gas concentrations (O<sub>2</sub>, CO<sub>2</sub> and N<sub>2</sub>) were measured every 20 ms using a QP9000 quadrupole mass spectrometer (CaSE) calibrated against precision-analysed gas mixtures. Gas was drawn continuously from the mouthpiece along the extended 45 ft sampling capillary line, which had a 5–95% rise time of < 80 ms and a delay of 2680 ms.

### Intramuscular high-energy phosphates determined by <sup>31</sup>P-MRS

The experiments were carried out in a 1.5 T super-conducting magnet (Signa Advantage, GE, Milwaukee, USA) with a 0.5 m bore using a one-pulse <sup>31</sup>P NMR acquisition. A surface coil (8 in transmit and 5 in receive), tuned to a frequency of 25.85 MHz for phosphorus, was placed under the quadriceps of the dominant leg (in all cases the right leg) midway between the knee and hip joint (Fig. 1). The coil was securely fastened to the table, and displacement of the leg over the coil was prevented by the broad non-elastic strap over the hips. This allowed knee extensor exercise to be performed with the volume of interest (VOI) always stationary with the leg in the relaxed position.

Prior to the data acquisition, a series of axial gradient recalled echo (GRE) images of the thigh were acquired to help optimize the radio frequency (RF) coil position. Shimming was then performed on the VOI using an automated shimming package provided by the manufacturers. The homogeneity of the magnetic field was

optimized by shimming to the proton signal of muscle water. The  $^{31}\text{P}$  RF excitation pulse was set at a level to give maximum [PCr] signals at a 5 s repetition rate from an 80 mm thick axial slice of muscle. FIDs for  $^{31}\text{P}$  spectra were collected every 1875 ms with a spectral width of 2500 Hz and 512 data points. Data were averaged every eight acquisitions and the dynamic signals for the three ATP peaks ( $\alpha$ ,  $\beta$  and  $\gamma$ ), PCr and  $\text{P}_i$  could be determined every 15 s during the rest-exercise-rest transition. This maximized the signal-to-noise ratio of the spectra and the time resolution of the data set. Signal intensities of each resonance were calculated (as a batch job) by means of the time-domain VARPRO fitting program (van der Veen *et al.* 1988), using the appropriate prior knowledge of the ATP multiplets (Stubbs *et al.* 1996).

As in the studies of Barstow *et al.* (1994*a,b*), Evans *et al.* (1997) and McCreary *et al.* (1996), the  $T_1$  (longitudinal relaxation time) saturation factor was assumed to remain constant for each resonance throughout the experiment, and all phosphate metabolite levels are presented relative to their pre-exercise control values.

### Analysis of [PCr] kinetics

Having established the areas of the three ATP peaks and the  $\text{P}_i$  and PCr peaks (Fig. 2) using the VARPRO fitting program, the data were converted to relative changes (% $\Delta$ ) as determined by their percentage relationships, using the average during the 240 s of controlled rest to establish the 100% level. Each rest-work transition was time aligned such that time zero was the onset of the constant-load exercise. The data set was then edited, such that any point that lay outside four standard deviations of the moving mean was considered to represent non-Gaussian 'noise' and deleted for kinetic analysis purposes. However, before any point was removed it was rechecked by re-running the VARPRO fitting program with the spectrum in question as a single task. The data set was then interpolated on a second-by-second basis and then corresponding data sets were superimposed and averaged. The [PCr] responses were then averaged over 10 s to give a mean value over the two or three exercise bouts performed for each subject.

The [PCr] response to the constant-load exercise was modelled as a monoexponential decline from the baseline to a new steady state in the form:

$$\Delta[\text{PCr}]_t = [\text{PCr}]_0 - \Delta[\text{PCr}]_{\text{ss}}(e^{-(t-\delta)/\tau}), \quad (1)$$

where  $[\text{PCr}]_0$  is the value of [PCr] at  $t = 0$ , and  $\Delta[\text{PCr}]_{\text{ss}}$  is the value to which the [PCr] declines below  $[\text{PCr}]_0$ . All data from time zero to the end of the exercise period (360 s) were included in the fitting process. This describes a first order response with  $\Delta[\text{PCr}]_{\text{ss}}$ ,  $\tau$  and  $\delta$  (delay) as the three defining parameters. In the case of the determination of [PCr] kinetics, the process was expected to begin with no delay and therefore  $\delta$  was constrained to zero, i.e. the start of exercise. When left unconstrained, however, the estimated  $\delta$  value was not significantly different from zero, varying by less than  $\pm 5$  s in all cases.

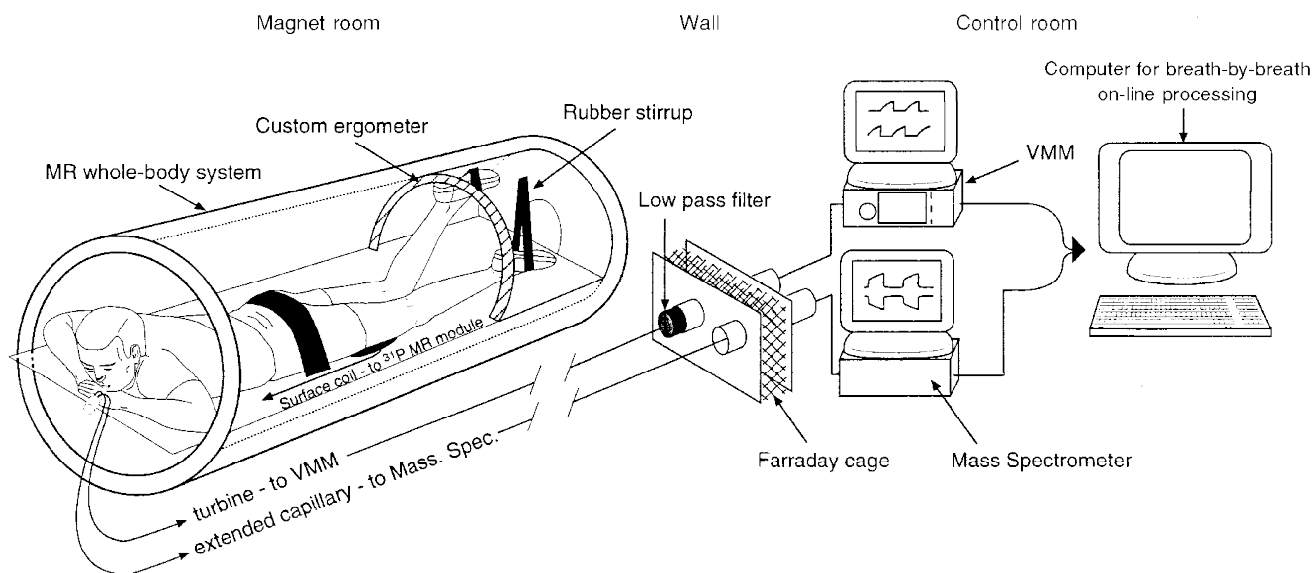
### Analysis of $\dot{V}_{\text{p},\text{O}_2}$ kinetics

The corresponding measurements of  $\dot{V}_{\text{p},\text{O}_2}$  were initially treated in the same way. Each data set was edited, as described above. However, no data points were removed from the phase I ( $\phi\text{I}$ ) region (i.e. that region associated with a 'cardiodynamic' increase in  $\dot{V}_{\text{p},\text{O}_2}$ ; Whipp *et al.* 1982). The data set was then interpolated on a second-by-second basis and then averaged every 10 s to provide a mean value for  $\dot{V}_{\text{p},\text{O}_2}$  for comparison with the simultaneously collected [PCr] data. The data were modelled in two ways. Firstly (model 1), the 'traditional' monoexponential response beginning at  $t = 0$  (i.e.  $\delta = 0$ ), where the whole data set was considered, thus:

$$\dot{V}_{\text{p},\text{O}_2,t} = \dot{V}_{\text{p},\text{O}_2,0} + \Delta\dot{V}_{\text{p},\text{O}_2,\text{ss}}(1 - e^{-t/\tau}), \quad 0 \leq t \leq 360. \quad (2)$$

This allows two parameters to be estimated,  $\Delta\dot{V}_{\text{p},\text{O}_2,\text{ss}}$  (change in steady-state  $\dot{V}_{\text{p},\text{O}_2}$ ) and  $\tau'$ , where  $\tau'$  is also termed the mean response time for  $\dot{V}_{\text{p},\text{O}_2}$ .

For the second fitting procedure (model 2), the data were again modelled as a single exponential but in this case the exponential component of the response was considered to begin at the phase I-phase II transition ( $\phi\text{I}-\phi\text{II}$  transition). The  $\phi\text{I}-\phi\text{II}$



**Figure 1.** Schematic representation of exercise in the NMR system

The  $^{31}\text{P}$  surface coil is placed under the quadriceps of the dominant leg and the subject breathes through the mouthpiece containing the tip of the extended sampling line and the non-ferrous turbine volume sensor. The output from the turbine passes through a low-pass filter, which is earthed to the Faraday cage, and into the volume measuring module (VMM).

transition was determined both by inspection (see Fig. 3) and by maximizing the prediction of  $\tau$  and its confidence limit. This means that the fitting field was moved around the expected  $\phi$ I- $\phi$ II transition and any change in  $\tau$ , or its associated confidence limit, was considered. As the fitting field was moved to include more 'non-exponential' data (moved closer to  $t=0$ ), the confidence limits of the prediction of  $\tau$  would be expected to increase. That is, if  $\phi$ I data were included in the fitting field, the predicted  $\tau$  would become greater and the confidence of the estimation would be reduced (i.e. the 95% confidence limits would increase). Thus, the  $\phi$ I- $\phi$ II transition could be determined to within the time resolution of the measurements, and the model 2 fit for the  $\dot{V}_{p,O_2}$  response to constant-load exercise can then be given by:

$$\dot{V}_{p,O_2,t} = \dot{V}_{p,O_2,0} + \Delta \dot{V}_{p,O_2,ss} (1 - e^{-(t-\delta)/\tau}), \quad \phi\text{I}-\phi\text{II} \leq t \leq 360. \quad (3)$$

The confidence limits for parameter estimation of  $\dot{V}_{p,O_2}$  and [PCr] can be described by eqn (6) (see Discussion). This gives us the ability to calculate the 95% confidence limits of each estimation of  $\tau$ , as described by Lamarra *et al.* (1987). The 'goodness-of-fit', however, is not characterized in the equations of Lamarra *et al.* (1987). The confidence limits for the prediction of  $\tau$ , therefore, will be expected to be within the limits described by eqn (6) only if the underlying process is a 'true' exponential. In order to determine how well the imposed function fitted the data, the residuals were considered both with respect to their flatness throughout the transient and by minimizing the sum squared errors (i.e.  $\chi^2$ ).

The functions were fitted to a non-linear least-squares regression using commercially available software (Microcal Origin). The predicted confidence limits of parameter estimation, considering the steady-state standard deviation of the noise, were determined by the equations of Lamarra *et al.* (1987). The actual confidence intervals associated with the imposed function were determined by

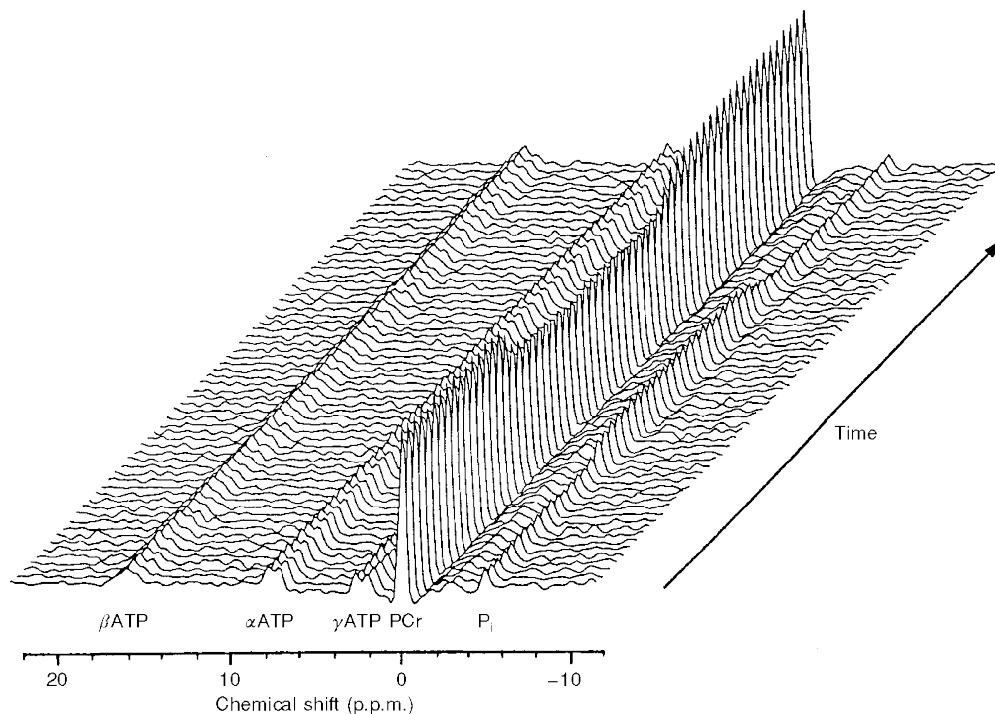
the fitting program; confidence was set at 95% and tolerance at 5% (i.e.  $P < 0.05$ ). The magnitude of the residuals was determined quantitatively by the  $\chi^2$  value.  $\tau_{\text{PCr}}$  for each subject was compared with  $\tau'_{\dot{V}_{p,O_2}}$  and  $\tau_{\dot{V}_{p,O_2}}$  as determined by the model 1 and model 2 functions, respectively. Values are given as means and standard deviations, or individual values and 95% confidence intervals, where indicated.

## RESULTS

### Intramuscular high-energy phosphate metabolism and [PCr] kinetics

An example of the 'stack plot' of the sequential spectra for subject 2 is given in Fig. 2. The areas under the peaks represent the relative concentrations of the three isoforms of ATP ( $\alpha$ ,  $\beta$  and  $\gamma$ ), PCr and  $P_i$ . The integrated relative concentration of all peaks of ATP did not change significantly at any point during the rest-exercise-rest protocol. An example of the response profile of [PCr] is shown in Fig. 3C. The response to the constant-load exercise was well described by a monoexponential (eqn (1)) with  $\delta = 0$  (i.e. with no delay).

The values for work rate approximation,  $\tau_{\text{PCr}}$  and  $\Delta[\text{PCr}]_{\text{ss}}$  for all six subjects are given in Table 1. The mean  $\Delta[\text{PCr}]_{\text{ss}}$  was  $-11.4\%$  ( $\pm 1.5$  s.d.); the mean  $\tau_{\text{PCr}}$  was 35 s ( $\pm 15.2$ ). All subjects expressed similar kinetic responses of [PCr] in response to the exercise apart from subject 3, who had a [PCr] response with a time constant significantly longer than that of other subjects.



**Figure 2.** The 'stack plot' of phosphorus metabolites in the exercising quadriceps from one representative subject (subject 2)

The reference peak, PCr, is set to 0 p.p.m. and each spectrum represents 15 s. The figure has been produced with the  $x$ -axis inverted such that the  $P_i$  peak may be visible in front of the large PCr peak.

Table 1. Comparison of the kinetic responses of  $\dot{V}_{p,O_2}$  and [PCr] to moderate intensity exercise

A.							
Subject no.	No. of transitions ( <i>n</i> )	~Work rate (W)	$\Delta[\text{PCr}]_{\text{ss}}$ (% $\Delta$ )	$\tau_{\text{PCr}}$ (s)	$K_{n\tau_{\text{PCr}}}$ (s)	Residual** (absolute %)	
1	2	80	13.3	35	3.1	0.786	
2	3	30	12.3	27	2.1	0.349	
3	2	30	8.8	64	4.6	0.454	
4	2	30	12.0	33	5.1	2.418	
5	1	30	11.0	20	3.5	2.081	
6	2	60	11.1	31	6.3	1.371	
Mean	2.0	43	11.4	35.0	4.1	1.2	
S.D.	0.6	22	1.5	15.2	1.5	0.86	

B.								
Subject no.	Model 1				Model 2			
	$\Delta \dot{V}_{p,O_2,ss}$ (ml min <sup>-1</sup> )	$\tau'_{\dot{V}_{p,O_2}}$ (s)	$K_{n\tau'}$ (s)	Residual** (absolute ml)	$\tau_{\dot{V}_{p,O_2}}$ (s)	$K_{n\tau}$ (s)	Residual** (absolute ml)	$\delta_{\dot{V}_{p,O_2}}$ (s)
1	820	54	2.5	0.0035	30	2.5	0.0016	25
2	320	54	1.8	0.0007	29	1.8	0.0002	26
3	530	92	4.6	0.0013	68	4.6	0.0008	15
4	260	52	3.1	0.0004	28	3.1	0.0003	29
5	220	57	4.7	0.0026	20	4.7	0.0012	33
6	650	64	3.0	0.0013	39	3.0	0.0010	22
Mean	467	62.2	3.28	0.0016	35.7	3.3	0.0085	25
S.D.	240	15.2	1.15	0.0012	17.0	1.2	0.0054	6.2

A, parameter estimates for [PCr] fall using eqn (1). B, parameter estimates for  $\dot{V}_{p,O_2}$  increase using model 1 (eqn (2)) and model 2 (eqn (3)). \* Delay ( $\delta$ ) for model 2  $\dot{V}_{p,O_2}$  only. \*\* Residuals expressed are  $\chi^2$  values. ~Work rate, work rate approximation (for calculation of work rate see Methods);  $\Delta x_{ss}$ , relative or absolute change in steady-state intramuscular [PCr] and  $\dot{V}_{p,O_2}$ ;  $\delta_{\dot{V}_{p,O_2}}$ , delay from start of exercise to the onset of the extrapolated exponential increase in  $\dot{V}_{p,O_2}$ ;  $\tau_x$ , time constant of intramuscular [PCr] decrease and  $\phi$ II  $\dot{V}_{p,O_2}$  increase;  $\tau'_{\dot{V}_{p,O_2}}$ , mean response time for  $\dot{V}_{p,O_2}$ ;  $K_{n\tau_x}$  and  $K_{n\tau'}$ , 95% confidence limits for the predictions of  $\tau$  and  $\tau'$  as defined by Lamarra *et al.* (1987).

$\tau_{\text{PCr}}$  for all subjects, except subject 3, lay within the range 20–35 s.  $\tau_{\text{PCr}}$  for subject 3, however, was significantly longer at 64 s. The monoexponential function provided a good description in all cases.

**$\dot{V}_{p,O_2}$  kinetic responses to moderate work**

The  $\dot{V}_{p,O_2}$  response to moderate work was, as expected, characterized by two temporal phases. The initial  $\phi$ I response contributed a small proportion of the entire response (range, 0–46%) and lasted between 15 and 33 s (Table 1). The subsequent  $\phi$ II response formed the majority of the response. In four of the subjects  $\dot{V}_{p,O_2}$  increased during  $\phi$ I; no increase was observed in two subjects (3 and 5).

The  $\Delta \dot{V}_{p,O_2,ss}$  was, as expected, significantly dependent on the imposed work rate approximation, with the subjects who were able to maintain the highest subthreshold work rate also reaching the highest  $\dot{V}_{p,O_2}$ , as shown in Table 1. It is salient to note, however, that subject 3 showed a greater

than expected increase in  $\dot{V}_{p,O_2}$  considering the work rate performed, presumably reflecting a low mechanical efficiency of accomplishing the exercise task.

The use of model 1 to estimate the mean response time for  $\dot{V}_{p,O_2}$  resulted in estimates of  $\tau'_{\dot{V}_{p,O_2}}$  that ranged from 52 to 92 s (Table 1). The expected 95% confidence intervals ( $K_{n\tau'}$ ) for parameter estimations for each subject are also given in Table 1. An example of the model 1 exponential fit, for the data set of subject 1, is shown in Fig. 3A. The monoexponential function with  $\delta=0$  clearly did not provide a good description of the actual  $\dot{V}_{p,O_2}$  response (Fig. 3A), particularly the early component. The systematic overestimation near the onset of exercise followed by the systematic underestimation of the subsequent response (in this case after approximately 75 s) is typical of the least-squares fitting of a two-compartment response with a single-compartment model (Whipp *et al.* 1982). This still allowed determination of the total O<sub>2</sub> deficit, however, as

**Table 2.** The oxygen deficit and estimated change in oxygen stores during the non-steady state of moderate exercise

Subject no.	~Work rate (W)	$\Delta \dot{V}_{p,O_2,ss}$ (ml min <sup>-1</sup> )	O <sub>2</sub> deficit $\Delta \dot{V}_{p,O_2,ss} \tau'$ (ml)	$\Delta[\text{O}_2 \text{ stores}] \dagger$ $\Delta \dot{V}_{p,O_2,ss} (\tau' - \tau)$ (ml)
1	80	820	738	328
2	30	320	277	123*
3	30	530	822	221*
4	30	260	225	104*
5	30	220	216	143*
6	60	650	704	282

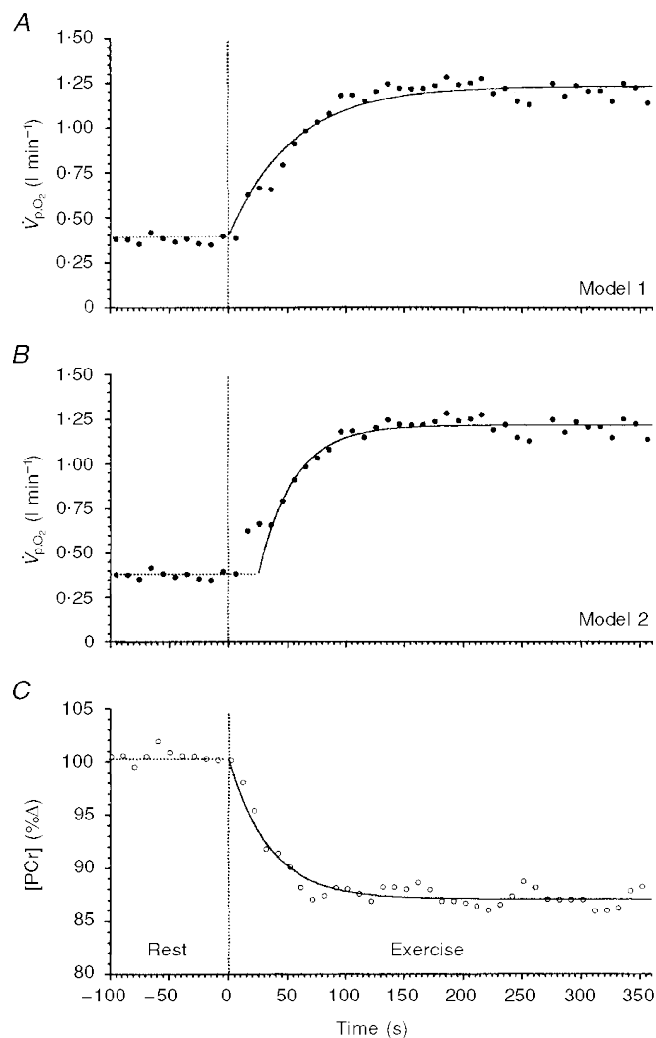
\* Similar to predicted values, as calculated from Whipp & Ward (1982). † For estimation of  $\Delta[\text{O}_2 \text{ stores}]$  see eqn (5) and Results. ~Work rate, work rate approximation (for calculation of work rate see Methods).  $\Delta \dot{V}_{p,O_2,ss}$ , absolute change in steady-state  $\dot{V}_{p,O_2}$ ;  $\tau'$ , mean response time for  $\dot{V}_{p,O_2}$ ;  $\tau$ , time constant for  $\phi$ II  $\dot{V}_{p,O_2}$  response.

the iterative process overestimated and underestimated the response to equal extents, and hence:

$$\text{O}_2 \text{ deficit} = \Delta \dot{V}_{p,O_2,ss} \tau'. \quad (4)$$

The values for the O<sub>2</sub> deficit are given in Table 2.

The second model, model 2, gave systematically smaller time constants for  $\dot{V}_{p,O_2}$ . The  $\tau_{\dot{V}_{p,O_2}}$  for  $\phi$ II only ranged between 20 and 39 s for all except subject 3 who, as with  $\tau_{\text{PCr}}$ , had a longer time constant of 68 s. The model 2 exponential fit to the data set of subject 1 is shown in Fig. 3*B*. This function



**Figure 3.** The responses of one representative subject (subject 1) to a constant-load change in work rate of a moderate intensity of  $\dot{V}_{p,O_2}$  and [PCr]

*A* and *B* show the same  $\dot{V}_{p,O_2}$  data modelled with different strategies; *C* shows the simultaneously determined [PCr]. The data in *A* are fitted with a monoexponential function beginning at the onset of exercise (model 1). The data in *B* are fitted with a monoexponential function that begins after the  $\phi$ I- $\phi$ II transition and does not include the  $\phi$ I response in the fitting procedure (model 2). The data in *C* are fitted with a monoexponential function constrained to begin at the onset of exercise.

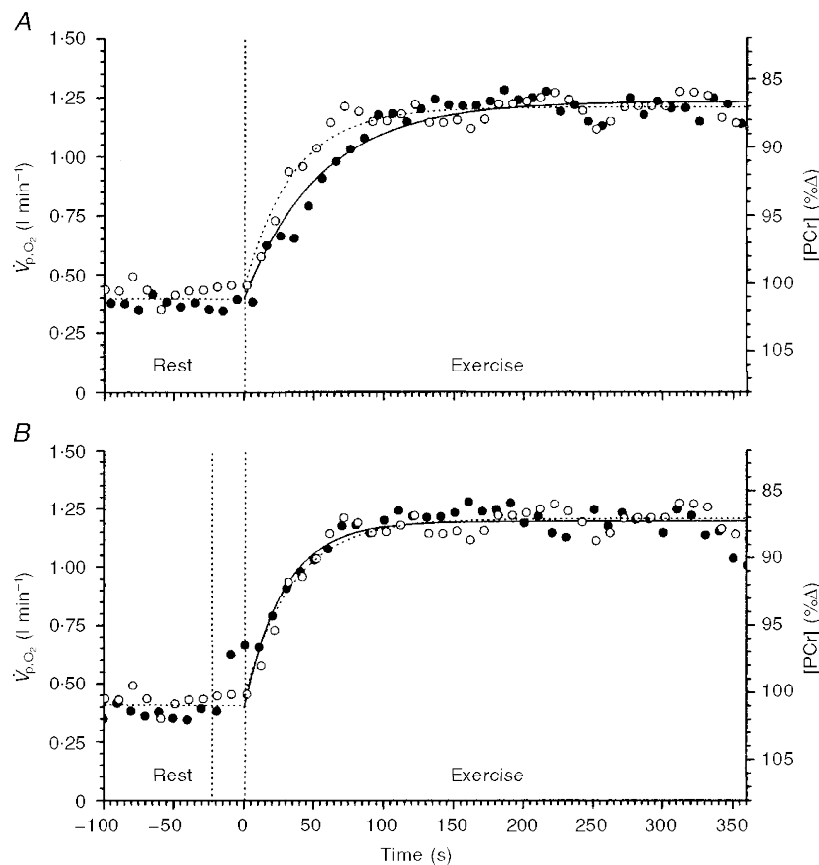
provided a good description of the  $\phi$ II response, as it did in all cases.

In order to compare the kinetics of the two variables, we phase-aligned the data. Firstly, the axes for [PCr] were inverted allowing us to compare the [PCr] fall with the  $\dot{V}_{p,O_2}$  increase. In the case of the model 1 fit, the start of exercise functioned as the start of the exponential for both [PCr] and  $\dot{V}_{p,O_2}$ . The two responses and their fitting functions for one representative subject are superimposed in Fig. 4A. Even by inspection, it is apparent that the two processes do not follow the same kinetic responses. For the model 2 fit (Fig. 4B), the data set first had to be phase aligned by moving the  $\dot{V}_{p,O_2}$  data set back in time such that the exponential functions began at the same point in time, i.e. ‘ignoring’ the  $\phi$ I data. This phase aligning of the [PCr] and  $\phi$ II  $\dot{V}_{p,O_2}$  responses to exercise allowed direct comparison of the simultaneously measured pulmonary and intramuscular variables. The two variables clearly follow the same time course. The original [PCr] fall and  $\dot{V}_{p,O_2}$  rise to their new steady-state levels for each subject are shown in Fig. 5A and B. The subsequent superimposition of the kinetic changes of [PCr] and  $\dot{V}_{p,O_2}$  (with the [PCr] axes inverted and the  $\dot{V}_{p,O_2}$  ‘phase shifted’)

for all six subjects are shown in Fig. 5C. The two variables appeared to follow the same dynamic response profile in all cases, and it is pertinent to point out that, even in the case of subject 3 who showed such slow  $\dot{V}_{p,O_2}$  kinetics, the time course of [PCr] was not discernibly different.

As shown in Fig. 6A, the time constants for  $\phi$ II  $\dot{V}_{p,O_2}$  lay very close to the line of identity with  $\tau_{PCr}$ . The error bars describe the 95% confidence limits of the estimation for the time constant for both variables. This suggested that  $\phi$ II  $\tau_{\dot{V}_{p,O_2}}$  closely reflected (i.e. to within 10%) the kinetics of muscular oxygen utilization ( $\dot{V}_{m,O_2}$ ) as previously proposed by Barstow *et al.* (1990) based on their computer modelling and by Grassi *et al.* (1996) using direct measurements of  $\dot{V}_{m,O_2}$ . The  $\tau'_{\dot{V}_{p,O_2}}$  given by model 1, however, systematically lengthened the estimated time constant for  $\dot{V}_{p,O_2}$  with respect to that of [PCr] (Fig. 6B).

Having determined the [PCr] kinetic response, it was possible to consider the sources of oxidative energy utilized during the transition. The difference in the mean response time for  $\dot{V}_{p,O_2}$  ( $\tau'$ , reflecting the entire oxygen consumption throughout the transient) and the  $\phi$ II time constant ( $\tau$ ,

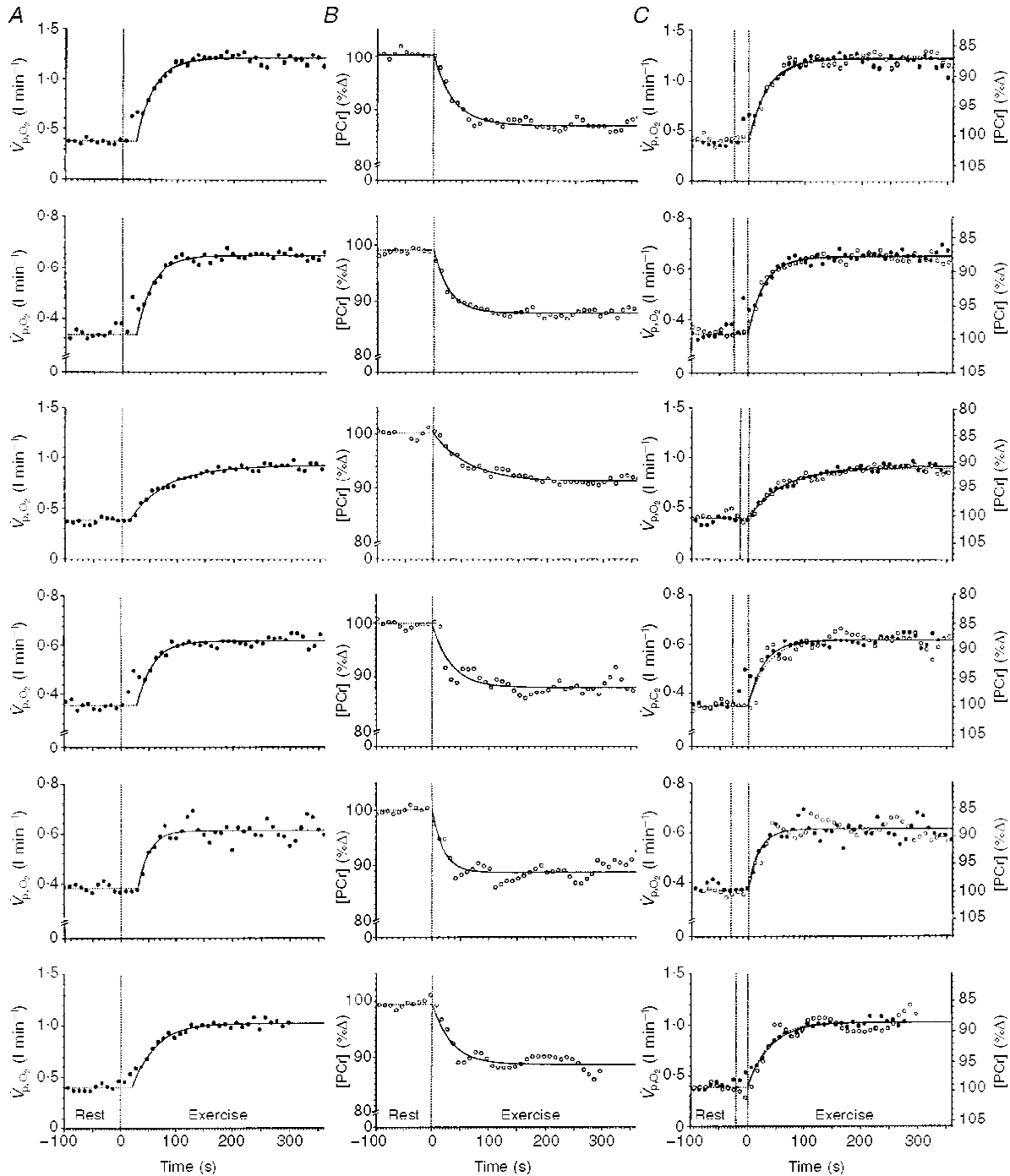


**Figure 4.** The responses of  $\dot{V}_{p,O_2}$  and [PCr] to a step change in work rate of moderate intensity for one subject (subject 1)

The responses are superimposed to show the dissimilarity, in the case of A, and similarity, in the case of B, of the kinetic responses of the two variables. A shows the  $\dot{V}_{p,O_2}$  (●) fitted by a model 1 exponential. B shows the  $\dot{V}_{p,O_2}$  (●) phase shifted and fitted by a model 2 exponential. The model 2 fit of  $\dot{V}_{p,O_2}$  closely represents the kinetic response of [PCr] (○) to the exercise (B).

reflecting muscular  $O_2$  utilization; Grassi *et al.* 1996) can be used to estimate the change in the oxygen stores, predominantly reflecting the change in muscular venous oxygen content ( $\Delta C_{v,O_2}$ ). Any reduction in oxygen dissolved in the muscle tissue water or from combination with myoglobin is likely to be minimal. Therefore, because cardiac

output ( $\dot{Q}$ ) and  $\dot{V}_{p,O_2}$  have a highly linear relationship in the steady state (Grimby *et al.* 1966; Rowell, 1969; Whipp *et al.* 1996) and with the assumptions that: (i)  $\tau_{PCR}$  is a surrogate of the time constant of  $\dot{V}_{m,O_2}$  ( $\tau_{\dot{V}_{m,O_2}}$ ; at these moderate work rates), (ii)  $\tau_{PCR} = \phi II \tau_{\dot{V}_{p,O_2}}$ , and (iii) the muscle blood flow and its distribution in prone exercise are comparable to those



**Figure 5.** The kinetic responses of  $\dot{V}_{p,O_2}$  and [PCr] to a constant-load change in work rate in six subjects

*A* shows  $\dot{V}_{p,O_2}$  with the  $\phi II$  response fitted by a monoexponential. *B* shows the simultaneously determined [PCr] response. *C* shows the identity of  $\phi II \dot{V}_{p,O_2}$  (●) and [PCr] (○) kinetics determined simultaneously during quadriceps exercise. The [PCr] scale has been inverted to facilitate kinetic comparisons. Graphs from top to bottom show data from subjects 1 to 6, respectively.



for upright cycling exercise, the change in oxygen stores (Whipp & Ward, 1982) can be estimated as:

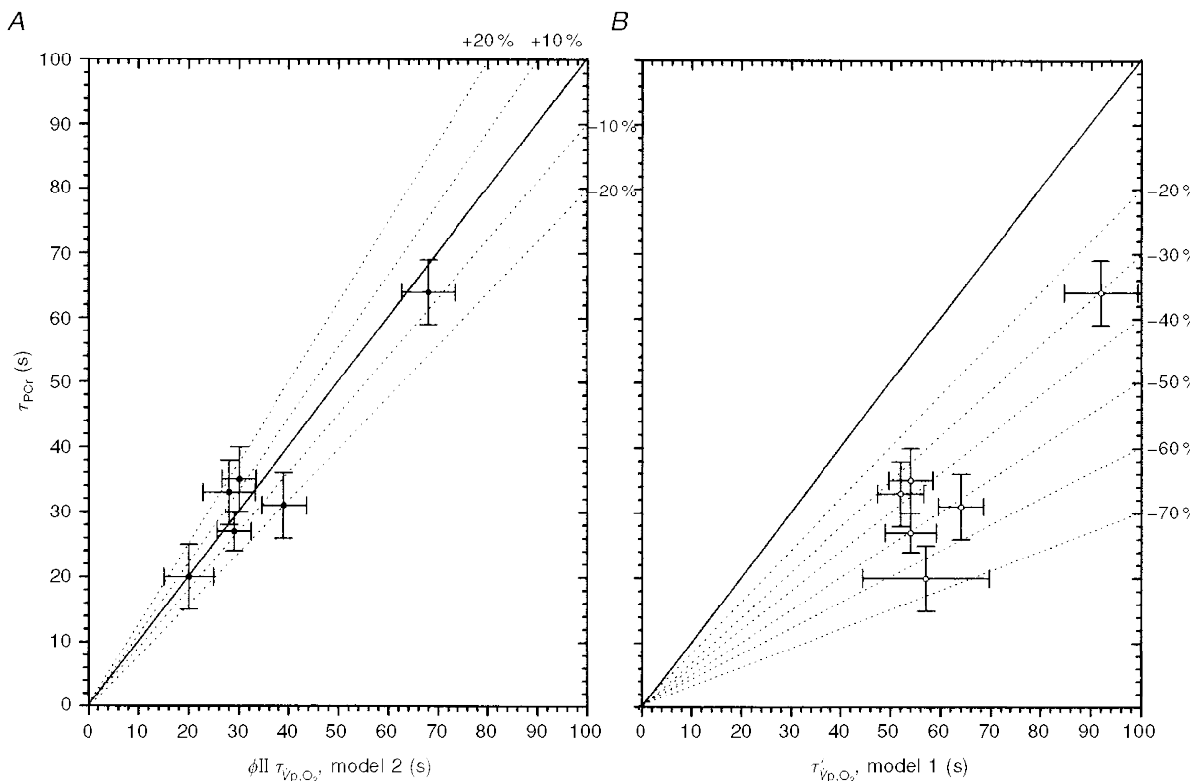
$$\Delta[\text{O}_2 \text{ stores}] = \Delta \dot{V}_{\text{p,O}_2, \text{ss}} (\tau' - \tau). \quad (5)$$

The values for the change in oxygen stores are given in Table 2. The values lay between 104 and 328 ml of O<sub>2</sub>. The prediction for  $\Delta[\text{O}_2 \text{ stores}]$  using this relationship would be expected to range from approximately 90 to 180 ml of O<sub>2</sub>, considering the work rates performed. Our estimates appear reasonable for the lower work rates but appear to be underestimates at the higher work rates (see Table 2). This is presumably due to the fact that our subjects were performing exercise transitions from rest. The assumption inherent in the equation of Whipp & Ward (1982) is that the relationship between  $\dot{V}_{\text{p,O}_2}$  and cardiac output is linear (with a positive intercept). However, this may not be the case when resting values are included. Furthermore, a greater contribution from transient increases in lactate ( $\Delta[\text{La}]$ ) at the higher absolute work rates is also possible.

### DISCUSSION

When measured over the relevant region, the kinetics of the  $\dot{V}_{\text{p,O}_2}$  and [PCr] responses lie within the expected equivalence bounds, i.e.  $\pm \sim 10\%$  (Figs 5C and 6, Table 1). That is, the  $\tau$  for  $\dot{V}_{\text{p,O}_2}$  during moderate exercise is likely to reflect the

average response of a heterogeneous ‘pool’ of muscle fibres with different contractile and biochemical characteristics (Kushmerick *et al.* 1992). The determination of the [PCr] fall in a relatively large section (approximately a hemisphere of diameter 5 in) of this exercising muscle (when compared with biopsy techniques, for example) effectively homogenizes the intramuscular response in an analogous sense to that for  $\dot{V}_{\text{m,O}_2}$  and  $\dot{V}_{\text{p,O}_2}$  measurements. Consequently,  $\dot{V}_{\text{p,O}_2}$  can be directly related to the ‘homogeneous’ [PCr] fall in the muscles performing the exercise. The temporal dissociation of  $\dot{V}_{\text{p,O}_2}$  from the intramuscular [PCr] fall, and by implication  $\dot{V}_{\text{m,O}_2}$ , is a necessary consequence of the size of the intervening venous volume and its blood flow dynamics. Studies by Mahler (1985) have shown a direct proportionality between the kinetics of [PCr] fall and  $\dot{V}_{\text{m,O}_2}$  in frog muscle, suggesting that our use of [PCr] as a proxy variable for the kinetics of muscle  $\dot{V}_{\text{O}_2}$  is justified, at least at moderate work rates. Furthermore, our results cohere well with those of Grassi *et al.* (1996) who demonstrated that the  $\tau$  of  $\dot{V}_{\text{m,O}_2}$  was not significantly different from that of  $\dot{V}_{\text{p,O}_2}$  at the same moderate intensity exercise; however, they did not measure [PCr]. It seems reasonable to assume, therefore, that the  $\phi \text{II}$   $\dot{V}_{\text{p,O}_2}$  response does indeed reflect that of intramuscular [PCr] fall, and by implication that of  $\dot{V}_{\text{m,O}_2}$ , during the transition from rest to moderate work in humans – at least to the limits of confidence provided by our data. While both



**Figure 6.** Comparisons of the time constants estimated by model 1 ( $\tau'$ ) and model 2 ( $\tau$ ) with the simultaneously determined  $\tau_{\text{PCr}}$

*A*, the time constants ( $\tau$ ) of  $\phi \text{II}$   $\dot{V}_{\text{p,O}_2}$  (model 2) and [PCr] lie within  $\sim 10\%$  of identity. The error bars give the 95% confidence intervals of the prediction of  $\tau$  for each subject. *B*, the mean response time of  $\dot{V}_{\text{p,O}_2}$ ,  $\tau'$  (model 1), systematically overestimates the time constant for the fall in muscle [PCr].

McCreary *et al.* (1996) and Barstow *et al.* (1994*a, b*) have also made this point, the small amplitude of the  $\Delta \dot{V}_{p,O_2}$  response ( $\sim 100 \text{ ml min}^{-1}$ ) in the former, and the different muscle groups operating over different ranges of  $\Delta \dot{V}_{p,O_2}$  in the latter (neither making comparisons of simultaneously acquired data), did not make these studies ideal for the purpose of inferring control mechanisms. The present study was designed to exercise a sufficiently large muscle mass to produce large changes in  $\Delta \dot{V}_{p,O_2,ss}$  and  $\Delta[\text{PCr}]_{ss}$  and for the two variables to be measured simultaneously (as have Evans *et al.* (1997) although not with reference to parameter estimation). These are important requirements which enable parameter estimation with the relevant level of confidence (approximately 10%; as discussed in Whipp *et al.* 1999). A further confounding influence on the parameter estimation is that of the breath-to-breath noise. This has previously been shown to be part of an uncorrelated Gaussian stochastic process thereby allowing parameter estimation to within known bounds of confidence. By increasing the number of exercise bouts performed, Lamarra *et al.* (1987) showed that the 95% confidence limits ( $K_n$ ) for the estimation of  $\tau$  can be increased. The limits of confidence can be described by the equation:

$$K_n = \hat{L} S_0 / \sqrt{(n \Delta x)}, \quad (6)$$

where  $x$  is either [PCr] or  $\dot{V}_{p,O_2}$ , assuming that the [PCr] 'noise' is similarly stochastic, as previously described for  $\dot{V}_{p,O_2}$ .  $\hat{L}$  is a constant depending on the value of the underlying  $\tau$  and therefore the number of data points through the early part of the transition.  $S_0$  is the standard deviation of the point-to-point fluctuation in the steady state (i.e.  $-240$  to  $0$  s at rest and approximately  $180$  to  $360$  s during exercise).  $n$  is the number of independent identical rest-work transitions that have been superimposed to minimize the influence of the stochastic noise process. The  $K_n$  for all predictions of  $\tau_{\text{PCr}}$  was within the limits of acceptability needed to make comparisons with the kinetic responses of  $\dot{V}_{p,O_2}$ .

The biphasic  $\dot{V}_{p,O_2}$  model proposed by Whipp *et al.* (1982), which has been used in the modelling of the  $\dot{V}_{p,O_2}$  responses in the present study, has important physiological implications, when compared with the more traditional models of Cerretelli & di Prampero (1987), Hill & Lupton (1923) and Whipp (1970). These authors describe the increase in pulmonary  $\dot{V}_{O_2}$  as a simple monoexponential function beginning at the onset of exercise. [PCr] breakdown, and any transient lactate production (as reflected in  $\Delta[\text{La}]$ ) (Cerretelli *et al.* 1979; Scheuermann *et al.* 1998), provides a buffer to the shortfall of oxygen uptake at the muscle. This traditional model of  $\dot{V}_{p,O_2}$  assumes that the contribution of the  $O_2$  stores to the energy transfer will result in an obligatory slowing of the  $\dot{V}_{p,O_2}$  kinetics relative to that of  $\dot{V}_{m,O_2}$ . The time constant for  $\dot{V}_{m,O_2}$  (and by implication [PCr]) is, in this model, obligatorily faster than the time constant of  $\dot{V}_{p,O_2}$ , as transient lactate production and a fall in venous oxygen stores causes a dissociation of the pulmonary and muscle  $\dot{V}_{O_2}$ . The greater the increment in work rate (i.e. the

greater the increment in  $\dot{V}_{p,O_2}$ ) the more pronounced is this dissociation.

The model of pulmonary oxygen uptake developed by Whipp *et al.* (1982), however, is based on the demonstration by Krogh & Lindhard (1913) that an initial phase of pulmonary  $\dot{V}_{O_2}$  is induced by the increase in blood flow through the lung ( $\dot{Q}$ ). This is followed by a subsequent, usually predominant, phase that is related to that of the muscle oxygen uptake to blood flow ratio ( $\dot{V}_{O_2}/\dot{Q}_m$ ) increase throughout the transient, resulting in a reduction of the muscle venous, and therefore mixed venous, oxygen content. Here the [PCr] change (or some function thereof), directly responsible for the characteristics of  $\dot{V}_{m,O_2}$ , will be closely reflected by the  $\phi\text{II } \dot{V}_{p,O_2}$  kinetics. Thus all three functions should manifest similar kinetics, i.e. to within approximately 10%, if the predictions of Barstow *et al.* (1990) are valid under these conditions. The provision of oxygen to the exercising muscle at the onset of exercise seems not to be a limiting factor at this moderate work intensity (Germino *et al.* 1996; Grassi *et al.* 1998), although Hughson and his associates (Hughson & Morrissey, 1982) have disputed this.

Having identified the relevant parameters and variables for comparison, a further complication is apparent. This is the potential of cardiodynamic 'distortion' of the  $\dot{V}_{m,O_2}$  kinetics on reaching the lung (Barstow *et al.* 1990). This potential distortion may interfere with the determination of any relationship between  $\tau_{\text{PCr}}$  and  $\tau_{\dot{V}_{p,O_2}}$ . For example, if at the onset of exercise cardiac output and muscle blood flow increased in precise proportion to  $\dot{V}_{m,O_2}$ , then no change in the  $O_2$  content of the venous effluent from the exercising muscle would be evident at any point during the transient. This increase would be immediately reflected in  $\dot{V}_{p,O_2}$  (i.e. entirely  $\dot{Q}$  dependent). It would therefore be identical to  $\dot{V}_{m,O_2}$ . In contrast, if  $\dot{Q}$  is constrained not to change throughout the transition, the increase in  $\dot{V}_{p,O_2}$  would only occur at a point in time *after* the onset of exercise, when the 'hypoxic' venous effluent reaches the lung. In this case, there would be an appreciable contribution of the  $O_2$  stores during the transient (see Fig. 2 of Whipp, 1994). The normal situation, where  $\dot{Q}$  does increase at the onset of exercise (Cummin *et al.* 1986), may confound any comparison of  $\tau_{\dot{V}_{p,O_2}}$  and  $\tau_{\text{PCr}}$ . The fall of [PCr] to a new steady-state level is expected to begin at the onset of exercise, but the blood flow associated with that fall also changes with its own kinetic response to exercise. This degree of the change in blood flow between the muscle and, some time later, the lung will be determined in large part by the kinetics of the increase in  $\dot{Q}$ . Consequently, if the kinetics of  $\phi\text{II } \dot{V}_{p,O_2}$  are determined by the kinetics of intramuscular [PCr] fall (or some function thereof), then any estimation of  $\tau_{\text{PCr}}$  would be expected to reflect  $\phi\text{II } \tau_{\dot{V}_{p,O_2}}$  only to within approximately 10% (Barstow *et al.* 1990).

The initial region ( $\phi\text{I}$ ) of the  $\dot{V}_{p,O_2}$  kinetics is dominated by the increase in blood flow at the onset of exercise (Whipp *et al.* 1982). The extent of the change in oxygen stores (i.e.  $\Delta C_{v,O_2}$ ,  $\Delta[\text{oxy-myoglobin}]$  and the  $O_2$  equivalent of

$\Delta[\text{La}]$ ) during the transient is related to the blood flow kinetics. More rigorously, the extent of the muscle oxygen stores utilization is directly proportional to the mean  $\dot{V}_{O_2}/\dot{Q}_m$  ratio at the exercising muscle. The immediacy of the fall of [PCr] at the onset of exercise suggests a similarly rapid increase in  $\dot{V}_{m,O_2}$ . This is contrary to the findings of Grassi *et al.* (1996, 1998) who suggest a pure delay dissociating the onset of an increase in  $\dot{V}_{m,O_2}$  from the onset of exercise. However,  $\dot{Q}_m$  does not increase in the same proportion as  $\dot{V}_{m,O_2}$  (or [PCr] fall), i.e.  $\dot{V}_{O_2}/\dot{Q}_m$  increases throughout the transient. The consequence of this is that the [PCr] decrement (or some function thereof), muscle oxygen consumption and  $\phi\Pi$  pulmonary oxygen uptake can all have approximately the same kinetics despite a fall in the body oxygen stores during the transition. The findings of this study, that  $\phi\Pi$   $\dot{V}_{p,O_2}$  and [PCr] kinetics are identical (to within  $\sim 10\%$ ), agree with the suggestions of this model. The differences in the necessary inferences between the model suggested by Whipp and colleagues (Whipp *et al.* 1982) (implying that the responses of [PCr],  $\dot{V}_{m,O_2}$  and  $\phi\Pi$   $\dot{V}_{p,O_2}$  will all have the same kinetics at moderate work rates) and those of the models of Hill (1926) and Cerretelli and colleagues (Cerretelli & di Prampero, 1987) (that necessitate different kinetics for these variables) relate directly to the model assumptions.

All subjects in the current study showed a biphasic response in  $\dot{V}_{p,O_2}$  to exercise, as expected. Subjects 1, 2, 4 and 6 all showed an increase in  $\dot{V}_{p,O_2}$  during the  $\phi I$  region, as expected, but subjects 3 and 5 appeared to have what would be better described as a pure delay in the  $\dot{V}_{p,O_2}$  response. Subject 3 also had very slow kinetics for  $\dot{V}_{p,O_2}$  and [PCr]; however,  $\tau_{\dot{V}_{p,O_2}}$  and  $\tau_{\text{PCr}}$  still agreed to within 10%. These two factors, slow kinetics and little or no  $\dot{V}_{p,O_2}$  increase during  $\phi I$ , combine to create a large  $O_2$  deficit. Furthermore, the  $\phi I$  profile is suggestive of a large contribution from the  $O_2$  stores to the energy transfer. The total  $O_2$  deficit of 822 ml is much greater than would be expected for an  $\sim 30$  W increase in work rate, presumably requiring a significant contribution from transient lactate production (Cerretelli *et al.* 1979). The long delay in  $\dot{V}_{p,O_2}$  for subject 5 gives rise to a large contribution of the  $O_2$  stores to the total  $O_2$  deficit. Using eqn (5), approximately 65% of the total  $O_2$  deficit appears to be due to the contribution from the  $O_2$  stores, reflecting the 'normal' oxygen uptake kinetics but a relatively long delay from the onset of exercise until the expression of increased muscle  $O_2$  extraction is evident at the lung (i.e.  $O_2$  store contribution to ATP synthesis would be greater). This value for the stored oxygen utilization is plausible considering the work rates and the kinetics of  $\dot{V}_{p,O_2}$ .

The results from the present study, that  $\phi\Pi$   $\tau_{\dot{V}_{p,O_2}}$  and  $\tau_{\text{PCr}}$  cohere to within  $\sim 10\%$ , are in agreement with those suggested by Barstow *et al.* (1990) from their computer modelling, and also with those of Grassi *et al.* (1996) who made direct measurements of muscle blood flow and muscle oxygen consumption during exercise. However, the assumptions of the Barstow model were related to upright exercise; we used prone exercise. We know of no data to

confirm the model assumptions for this mode of exercise, i.e. the size and constancy of the venous volume and the dynamics of the increases in  $\dot{Q}$  and  $\dot{Q}_m$ . The present results demonstrate that  $\tau'_{\dot{V}_{p,O_2}}$ , estimated by model 1, systematically overestimates the time constant for  $\dot{V}_{p,O_2}$  or [PCr] due to the influence of the  $\phi I$  component on the fitting procedure. Consequently, the conclusion that  $\dot{V}_{p,O_2}$  kinetics are necessarily slower than  $\dot{V}_{m,O_2}$  kinetics is, we believe, incorrect. However, this model 1 time constant, i.e. the mean response time ( $\tau'_{\dot{V}_{p,O_2}}$ ), is physiologically useful; it can be validly used to establish the magnitude of the  $O_2$  deficit. The difference between  $\tau$  and  $\tau'$ , therefore, reflects the relative contribution of the  $O_2$  stores and any transient lactate production to ATP synthesis during the non-steady state.

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