

# Dynamic asymmetry of phosphocreatine concentration and O<sub>2</sub> uptake between the on- and off-transients of moderate- and high-intensity exercise in humans

H. B. Rossiter\*, S. A. Ward†, J. M. Kowalchuk\*‡, F. A. Howe§, J. R. Griffiths§ and B. J. Whipp\*

Departments of \*Physiology and §Biochemistry, St George's Hospital Medical School, Cranmer Terrace, Tooting, London, UK, †Centre for Exercise Science and Medicine, University of Glasgow, UK and ‡Canadian Centre for Activity and Ageing, University of Western Ontario, London, Ontario, Canada

The on- and off-transient (i.e. phase II) responses of pulmonary oxygen uptake ( $\dot{V}_{O_2}$ ) to moderate-intensity exercise (i.e. below the lactate threshold,  $\theta_L$ ) in humans has been shown to conform to both mono-exponentiality and 'on-off' symmetry, consistent with a system manifesting linear control dynamics. However above  $\theta_L$  the  $\dot{V}_{O_2}$  kinetics have been shown to be more complex: during high-intensity exercise neither mono-exponentiality nor 'on-off' symmetry have been shown to appropriately characterise the  $\dot{V}_{O_2}$  response. Muscle [phosphocreatine] ([PCr]) responses to exercise, however, have been proposed to be dynamically linear with respect to work rate, and to demonstrate 'on-off' symmetry at all work intensities. We were therefore interested in examining the kinetic characteristics of the  $\dot{V}_{O_2}$  and [PCr] responses to moderate- and high-intensity knee-extensor exercise in order to improve our understanding of the factors involved in the putative phosphate-linked control of muscle oxygen consumption. We estimated the dynamics of intramuscular [PCr] simultaneously with those of  $\dot{V}_{O_2}$  in nine healthy males who performed repeated bouts of both moderate- and high-intensity square-wave, knee-extension exercise for 6 min, inside a whole-body magnetic resonance spectroscopy (MRS) system. A transmit–receive surface coil placed under the right quadriceps muscle allowed estimation of intramuscular [PCr];  $\dot{V}_{O_2}$  was measured breath-by-breath using a custom-designed turbine and a mass spectrometer system. For moderate exercise, the kinetics were well described by a simple mono-exponential function (following a short cardiodynamic phase for  $\dot{V}_{O_2}$ ), with time constants ( $\tau$ ) averaging:  $\tau\dot{V}_{O_2, \text{on}}$   $35 \pm 14$  s ( $\pm$  s.d.),  $\tau[\text{PCr}]_{\text{on}}$   $33 \pm 12$  s,  $\tau\dot{V}_{O_2, \text{off}}$   $50 \pm 13$  s and  $\tau[\text{PCr}]_{\text{off}}$   $51 \pm 13$  s. The kinetics for both  $\dot{V}_{O_2}$  and [PCr] were more complex for high-intensity exercise. The fundamental phase expressing average  $\tau$  values of  $\tau\dot{V}_{O_2, \text{on}}$   $39 \pm 4$  s,  $\tau[\text{PCr}]_{\text{on}}$   $38 \pm 11$  s,  $\tau\dot{V}_{O_2, \text{off}}$   $51 \pm 6$  s and  $\tau[\text{PCr}]_{\text{off}}$   $47 \pm 11$  s. An associated slow component was expressed in the on-transient only for both  $\dot{V}_{O_2}$  and [PCr], and averaged  $15.3 \pm 5.4$  and  $13.9 \pm 9.1$  % of the fundamental amplitudes for  $\dot{V}_{O_2}$  and [PCr], respectively. In conclusion, the  $\tau$  values of the fundamental component of [PCr] and  $\dot{V}_{O_2}$  dynamics cohere to within 10 %, during both the on- and off-transients to a constant-load work rate of both moderate- and high-intensity exercise. On average, ~90 % of the magnitude of the  $\dot{V}_{O_2}$  slow component during high-intensity exercise is reflected within the exercising muscle by its [PCr] response.

(Received 27 June 2001; accepted after revision 25 March 2002)

**Corresponding author** B. J. Whipp: Centre for Exercise Science and Medicine, West Medical Building, Glasgow University, Glasgow G12 8QQ, UK. Email: brianwhipp@btopenworld.com

Current considerations of the on- and off-transient (i.e. phase II) responses of pulmonary oxygen uptake ( $\dot{V}_{O_2}$ ) to moderate-intensity exercise (i.e. below the lactate threshold,  $\theta_L$ ) in humans suggest that the response manifests both mono-exponentiality and 'on-off' symmetry (Linnarsson, 1974; Paterson & Whipp, 1991; Özyener *et al.* 2001); i.e. consistent with a system manifesting linear control dynamics (e.g. Milsum, 1966; Lamarra *et al.* 1983).

Above  $\theta_L$ , however, the  $\dot{V}_{O_2}$  response is more complex (Whipp & Wasserman, 1972; Linnarsson, 1974; Hughson & Morrissey, 1982; Barstow & Molé, 1991; Paterson &

Whipp, 1991) with the on-transient supra- $\theta_L$   $\dot{V}_{O_2}$  response consisting of two main components: (1) a 'fundamental' phase (phase II) well described by an exponential, and (2) a subsequent delayed phase yielding a slowly developing supplemental rise in  $\dot{V}_{O_2}$  (e.g. Barstow & Molé, 1991; Paterson & Whipp, 1991), which has been termed 'excess'  $\dot{V}_{O_2}$  or the  $\dot{V}_{O_2}$  slow component ( $\dot{V}_{O_2, \text{sc}}$ ) (Whipp, 1987). That is, neither mono-exponentiality nor 'on-off' symmetry characterise the  $\dot{V}_{O_2}$  response (Linnarsson, 1974; Cerretelli *et al.* 1977; Paterson & Whipp, 1991; Gerbino *et al.* 1996; MacDonald *et al.* 1997; Bohnert *et al.* 1998; Langsetmo & Poole, 1999; Özyener *et al.* 2001).

However, the dynamics of putative phosphate-linked controllers of muscle oxygen consumption ( $\dot{V}_{O_2}$ ), such as intramuscular [phosphocreatine] ([PCr]) (e.g. Meyer, 1988), have received far less attention. Consequently, it is not known whether the asymmetries in the  $\dot{V}_{O_2}$  responses to different exercise intensities are also evident in these intramuscular responses, or whether the dynamic 'on-off'  $\dot{V}_{O_2}$  asymmetry (i.e. with the slow component being evident at the on- but not, or much less prominent at, the off-transient; Paterson & Whipp, 1991; Özyener *et al.* 2001) is a consequence of similar features in intramuscular [PCr] profiles.

Although standard biopsy techniques have allowed [PCr] and levels of related high-energy phosphate metabolites to be determined at discrete points during the transient and steady-states of exercise (e.g. Piiper *et al.* 1968; Piiper & Spiller, 1970; Holloszy *et al.* 1971; Crow & Kushmerick, 1982; Bangsbo, 1996), the high temporal resolution provided by  $^{31}\text{P}$  magnetic resonance spectroscopy ( $^{31}\text{P}$ -MRS) has allowed robust kinetic characterisations of the intramuscular responses for both the on- and off-transients (e.g. Meyer, 1988; Kushmerick *et al.* 1992; Conley *et al.* 2001; Jubrais *et al.* 2001). Muscle [PCr] responses to exercise have been reported to be dynamically linear with respect to work rate (Meyer, 1988; Barstow *et al.* 1994), including 'on-off' symmetry (Meyer, 1988). However, the [PCr] and  $\dot{V}_{O_2}$  responses to exercise of different intensities have not been determined in concert nor with sufficient sensitivity to discriminate possible small, but real differences between their kinetics. We were therefore interested in examining: (1) the degree to which the different kinetic characteristics of the  $\dot{V}_{O_2}$  responses to moderate- and high-intensity square-wave exercise reflected those of the simultaneously measured [PCr] responses, and (2) the relationship between the on- and off-transient kinetic responses of  $\dot{V}_{O_2}$  and [PCr]. We elected to make repeated measurements of the variables in order to provide high-confidence estimation of the kinetic parameters (Lamarra *et al.* 1987; Rossiter *et al.* 2000).

## METHODS

The experimental methods are summarised here, having been previously described by Whipp *et al.* (1999) and Rossiter *et al.* (1999).

### Subjects

Nine healthy male volunteers with average age of 26 years (range 20–58 years) provided informed consent to participate in the study (as approved by the Local Research Ethics Committee for Human Experimentation in accordance with the Declaration of Helsinki). The subjects' characteristics are given in Table 1.

### Exercise protocols

During comprehensive habituation tests in the Laboratory of Human Physiology: (a) subjects were familiarised, and (b) moderate- and high-intensity work rates were identified, i.e. demarcation of  $\dot{V}_{O_2}$  responses either conforming to mono-

exponential characteristics (moderate) or associated with a slow phase of  $\dot{V}_{O_2}$  kinetics typically leading to fatigue after ~10 min (high).

Simultaneous determination of pulmonary  $\dot{V}_{O_2}$  and intramuscular [PCr] was made from 70 experiments (an average of eight visits by each subject) with the subjects lying prone inside the bore of the 1.5 Tesla super-conducting magnet (Signa Advantage, GE, Milwaukee, USA) with their feet suspended in the rubber stirrups of a custom-designed ergometer (Whipp *et al.* 1999). The rubber stirrups were tailored to each subject to permit either moderate- or high-intensity square-wave exercise by means of rhythmic alternate-leg knee-extensions (32 pulses  $\text{min}^{-1}$  for each leg). The required work rates for each subject were estimated (~ $\dot{W}$ ) by the methods of Whipp *et al.* (1999) (Table 2). The contraction phase of the knee extensors of the non-dominant leg occurred in unison with the  $^{31}\text{P}$ -MRS acquisition of the quadriceps of the relaxed dominant leg. The subject was 'strapped down' to the scanner table by means of a broad non-distensible strap placed over the hips throughout, to minimise extraneous movements.

Subjects performed between three and five repetitions (each on a different day) of both moderate- and high-intensity exercise in a square-wave exercise protocol consisting of 4 min at rest, 6 min of exercise at the pre-determined level, and then 6 min rest (recovery). The number of repeats required for each subject was governed by the extent to which increasing test repeats improved the overall signal-to-noise characteristics of the averaged  $\dot{V}_{O_2}$  and [PCr] responses, therefore allowing appropriate convergence and confidence limits (Rossiter *et al.* 2000) for the subsequent parameter estimation procedures.

### $^{31}\text{P}$ -MRS sequence

A one-pulse  $^{31}\text{P}$ -MRS acquisition was employed using a surface coil tuned to a frequency of 25.85 MHz for phosphorus, placed under the quadriceps muscle of the dominant leg (in all cases the right leg). The coil was securely fastened to the table. Prior to the  $^{31}\text{P}$  data acquisition, a series of axial gradient-recalled echo images of the thigh were acquired. Shimming was performed with an automated shimming package to the proton signal of muscle water (GE, Milwaukee, USA). The  $^{31}\text{P}$ -radio frequency excitation pulse was set at a level to give maximum [PCr] signals at a 5 s repetition rate from a 80 mm thick axial 'slice' of muscle. Throughout the entire test protocol (rest–exercise–recovery), free induction decays (FIDs) for  $^{31}\text{P}$  spectra were collected every 1875 ms with a spectral width of 2500 Hz and 512 data points.  $^{31}\text{P}$ -MRS data were averaged over eight acquisitions to estimate every 15 s the relative signal intensities of the three ATP peaks ( $\alpha$ ,  $\beta$  and  $\gamma$ ), PCr and  $\text{P}_i$ .

Signal intensities of each resonance were calculated (as a batch job) by means of the time-domain variable-projection fitting program, VARPRO (van der Veen *et al.* 1988), using the appropriate prior knowledge of the ATP multiplets (Stubbs *et al.* 1996). The  $T_1$  saturation factor was assumed to remain constant for each resonance throughout the experiment. Intramuscular pH was estimated from the relative chemical shift of the  $\text{P}_i$  peak towards the PCr peak:

$$\text{pH} = 6.75 + \log((\delta - 3.27)/(5.69 - \delta)), \quad (1)$$

where  $\delta$  is the relative chemical shift of [ $\text{P}_i$ ] to [PCr] (Moon *et al.* 1973).

### Pulmonary gas exchange measurement

$\dot{V}_{O_2}$  responses were determined breath-by-breath (Clinical and Scientific Equipment, CaSE, Gillingham, Kent, UK) (Beaver *et al.*

**Table 1. Subject characteristics**

Subject	Age (years)	Height (cm)	Weight (kg)
1	24	178	75
2	25	183	95
3	22	186	90
4	58	185	86
5	20	196	97
6	21	187	80
7	21	180	70
8	23	185	81
9	24	181	71
Mean	26	185	83
S.D.	11.9	5.2	9.9

1973; Whipp *et al.* 1999). Inspired and expired volume were measured by a bi-directional turbine (VMM, Interface Associates, Laguna Niguel, CA, USA), calibrated with a 3 l syringe over a range of flows (Hans Rudolph, Kansas City, MO, USA). The concentrations of respired gases ( $O_2$ ,  $CO_2$  and  $N_2$ ) were measured every 20 ms using a quadrupole mass spectrometer (QP9000, CaSE, Gillingham, Kent, UK), which was calibrated against precision-analysed gas mixtures. Respired gas was drawn continuously from the mouthpiece along the extended 45 ft capillary sampling line to the mass spectrometer, yielding a transit delay of 1.9 s and a 5–95 % rise time of < 80 ms (e.g. Whipp *et al.* 1999).

### Kinetic analysis

The kinetic features of both the  $\dot{V}_{O_2}$  and [PCr] responses to moderate- and high-intensity exercise were estimated in a similar fashion. There were, however, a few key differences. The [PCr] data were initially converted to relative changes established from the resting baseline (% $\Delta$ ), the value of the resting control phase being taken as 100 %. Both the  $\dot{V}_{O_2}$  and [PCr] responses have been shown to conform to the structure of a 'pure' underlying response superimposed with an uncorrelated 'noise' function that is Gaussian and independent of metabolic rate (Lamarra *et al.* 1987; Rossiter *et al.* 2000). This not only allows our editing procedure (Lamarra *et al.* 1987; Rossiter *et al.* 2000) to eliminate those data lying outside 4 standard deviations of the local mean (arising from coughs, sighs or swallows in the case of  $\dot{V}_{O_2}$  or associated to the inherently poor signal-to-noise characteristics of [PCr] measurement by  $^{31}P$  MRS), but also provides the justification for averaging the responses of like-exercise transitions for each subject to reduce the influence of the 'noise' on the subsequent parameter estimation. Thus the like-exercise  $\dot{V}_{O_2}$  and [PCr] responses were time-aligned to the onset of exercise (corresponding to time zero), interpolated on a second-by-second basis, and then superimposed and averaged for each subject; the  $\dot{V}_{O_2}$  responses were averaged every 10 s, and the [PCr] responses every 15 s (i.e. at their MRS sample frequency).

The resulting  $\dot{V}_{O_2}$  and [PCr] responses were modelled with non-linear least-squares fitting procedures (Microcal, Origin) to an exponential response, with the duration, time window and delay characteristics of the fitting being dependent on both the variable and the exercise intensity.

The on-transients of both variable responses to moderate-intensity exercise were modelled as a mono-exponential: for [PCr], beginning at time zero and falling to a new steady state; for  $\dot{V}_{O_2}$ , beginning

after the 'cardiodynamic' (or phase I) duration and increasing to a new steady state (Whipp *et al.* 1982):

$$\dot{V}_{O_2(t)} = \dot{V}_{O_2,0} + \Delta\dot{V}_{O_2,ss} (1 - e^{-(t-\delta)/\tau}), \quad (2)$$

and

$$\Delta PCr_{(t)} = PCr_0 - \Delta PCr_{ss} (1 - e^{-t/\tau}), \quad (3)$$

where  $\dot{V}_{O_2,0}$  and  $PCr_0$  are the values of  $\dot{V}_{O_2}$  and [PCr] at  $t = 0$ ,  $\Delta\dot{V}_{O_2,ss}$  and  $\Delta PCr_{ss}$  are the asymptotic values to which  $\dot{V}_{O_2}$  and [PCr] project,  $\tau$  is the time constant of the responses and  $\delta$  is a delay term similar to (but not equal to), the phase I – phase II transition term (Whipp *et al.* 1982; Whipp & Özyener, 1998). (N.B. the phase I, or 'cardiodynamic' region of the  $\dot{V}_{O_2}$  response has been shown to arise from increased lung perfusion (Whipp *et al.* 1982; cf. Krogh & Lindhard, 1913) and is thought not to be related to muscle  $O_2$  consumption; the subsequent phase II region has been demonstrated to be related to the arrival at the lung of the venous effluent from the exercising muscle (Casaburi *et al.* 1979; Grassi *et al.* 1996) and is therefore considered the relevant region for comparison with the intramuscular [PCr] dynamics.) As the corresponding  $\delta$  term for [PCr] has been shown not to differ significantly from zero, it was not included in the modelling process (Rossiter *et al.* 1999). Similar models for the off-transient were used:

$$\dot{V}_{O_2(t)} = \dot{V}_{O_2,0} - \Delta\dot{V}_{O_2,ss} (1 - e^{-(t-\delta)/\tau}), \quad (4)$$

and

$$\Delta PCr_{(t)} = PCr_0 + \Delta PCr_{ss} (1 - e^{-t/\tau}). \quad (5)$$

The confidence limits for the parameter estimation (Rossiter *et al.* 2000) and the  $\chi^2$  value were also obtained; confidence was set at 95 % and tolerance at 5 % (i.e.  $P \leq 0.05$ ).

In the case of high-intensity exercise, the fitting strategy was designed to identify, *a posteriori*, the onset of a putative delayed 'slow component' in the  $\dot{V}_{O_2}$  and [PCr] responses. The entire 6 min were therefore not automatically included in the data field for the fit. Rather, the fitting window extended from exercise onset (i.e.  $t = 0$  s for [PCr];  $t =$  time at the end of phase I for  $\dot{V}_{O_2}$ ) initially only 60 s into the exercise. The window was lengthened iteratively, until the exponential model fit demonstrated a discernible and consistent departure from the measured response profiles. However, as the goodness-of-fit cannot be extracted using the approach of Lamarra *et al.* (1987), two alternative indices were used: (1) the maintenance of the flat profile of the residual plot (i.e. signifying a good fit to the measured data), as judged by visual inspection, and (2) the demonstration of a local 'threshold' for increase in the  $\chi^2$  value. Once the exponential model fit diverged from the measured data, the fitting field was moved back in time by one datum to isolate the fundamental component of the response and the best-fit exponential was then established (see above). This gave extrapolated steady-state values for [PCr] and  $\dot{V}_{O_2}$  that were above and below (respectively) the final value actually attained. The magnitude of the  $\dot{V}_{O_2}$  and [PCr] slow components ( $\dot{V}_{O_2,sc}$  and  $[PCr]_{sc}$ ) were taken as the difference between the amplitude of the final measured value (relative to the pre-exercise resting baseline, averaged for the last 30 s) ( $\Delta\dot{V}_{O_2,end}$ ,  $\Delta[PCr]_{end}$ ) and the estimated steady-state amplitude of the fundamental ( $\Delta\dot{V}_{O_2,ss}$  and  $\Delta[PCr]_{ss}$ ). The percentage contribution of the slow component to the total response of variable  $y$  thus equals:  $((\Delta y_{end} - \Delta y_{ss})/\Delta y_{ss}) \times 100$ , where  $y$  is  $\dot{V}_{O_2}$  or [PCr].

The differences between parameter values were examined by repeated-measures ANOVA, across intensity (moderate vs. high), transition (on- vs. off-transition) and by variable ( $\dot{V}_{O_2}$  vs. PCr). Significance of all the fits to the responses were also estimated using the  $\chi^2$  goodness-of-fit test (Huntsberger, 1961). Values are

given as means and standard deviations, or 95 % confidence intervals ( $C_{95}$ ) where indicated; a threshold  $P$  value of less than 0.05 was used to establish significance.

## RESULTS

### Moderate-intensity exercise

A representative stack plot of the sequential MR spectra is given in Fig. 1A. The relative concentrations of the three phosphoryl residues of ATP ( $\alpha$ ,  $\beta$  and  $\gamma$ ) remained constant throughout the on- and off-transients while [PCr] declined to a new steady-state level after approximately 3 min of exercise. The temporal response  $\dot{V}_{O_2}$  and [PCr] profiles (for subject 4) for the moderate-intensity (and high-intensity) on-transitions are shown in Fig. 2. An early cardiodynamic region (phase I) was evident for the  $\dot{V}_{O_2}$  response but not for [PCr] at both moderate- and high-intensity exercise.

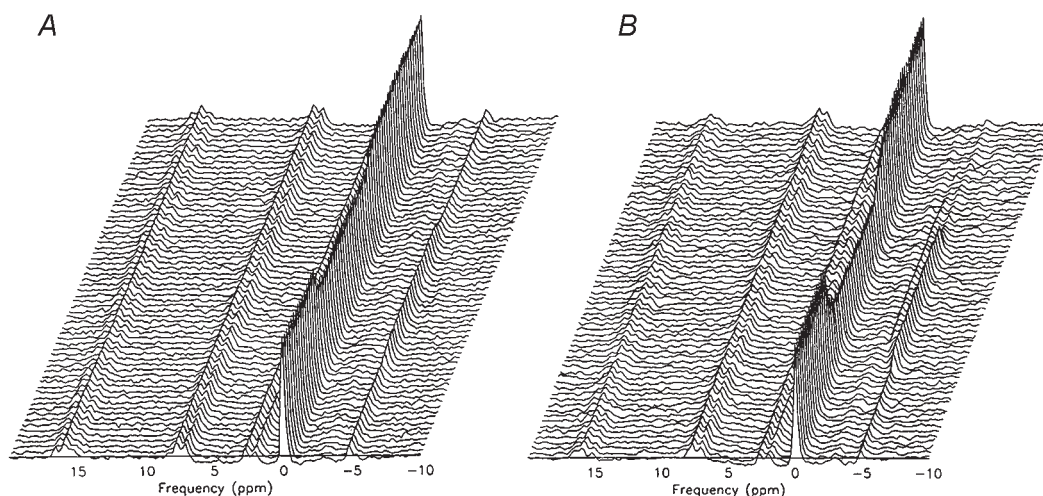
Mono-exponential modelling of the fundamental response (i.e. the entire 6 min response for [PCr], and following the phase I region for  $\dot{V}_{O_2}$ ) provided an adequate characterisation of the responses (Fig. 2A) in every case. The individual steady-state  $\dot{V}_{O_2}$  increments ( $\Delta\dot{V}_{O_2,ss}$ ) were, as expected, dependent on the work rate ( $\dot{W}$ ) performed, with those subjects who were able to maintain the highest subthreshold work rate also reaching the highest  $\dot{V}_{O_2}$  (Table 2). This, however, was not always the case with the PCr response ( $\Delta[\text{PCr}]_{ss}$ ), possibly reflecting differences in the resting absolute [PCr] concentrations within the region of interest across subjects and/or differences in the underlying  $\tau$  for oxidative metabolism. For the group as a whole,  $\Delta\dot{V}_{O_2,ss}$  averaged  $420 \pm 200 \text{ ml min}^{-1}$  and  $\Delta[\text{PCr}]_{ss}$  was  $11.1 \pm 2.3\%$  (Fig. 2A; Table 2). The mean  $\Delta\text{PCr}_{ss}$  ( $11.1 \pm 2.6\%$ ) and  $\Delta\dot{V}_{O_2,ss}$  ( $430 \pm 200 \text{ ml min}^{-1}$ ) at the off-transient, were, as

expected, not significantly different from those measured at the on-transient; i.e. both [PCr] and  $\dot{V}_{O_2}$  recovered to, or close to, 100 % of the pre-exercise baseline values within 6 min, in all cases.

The on-transient time constants for the individual responses averaged  $33 \pm 12$  and  $35 \pm 14$  s for [PCr] and  $\dot{V}_{O_2}$ , respectively, and their associated confidence intervals ( $C_{95}$ ) were less than  $\pm 3$  s (Table 3). Similarly, the off-transient kinetics were also well characterised by a mono-exponential function (see below, Fig. 4); however, in all but one case, the off-transient time constants were significantly longer than the corresponding on-transient  $\tau$ , both for [PCr] and  $\dot{V}_{O_2}$  ( $P < 0.05$ ). The off-transient  $\tau$  values averaged  $51 \pm 13$  and  $50 \pm 13$  s for [PCr] and  $\dot{V}_{O_2}$ , respectively (Table 3). Again, the narrow  $C_{95}$  bands illustrated the high confidence of the parameter estimation ( $C_{95}$  averaged  $\pm 2.6$  s). Despite the differences observed between the on- and off-transient  $\tau$  values for  $\dot{V}_{O_2}$  and [PCr], the on-transient  $\tau\dot{V}_{O_2}$  and  $\tau[\text{PCr}]$  were not statistically different; this was also the case for the off-transient (Table 3).

### High-intensity exercise

An example of the 'stack plot' of the sequential spectra for subject 2 is shown in Fig. 1B. As with moderate exercise, [ATP] remained constant throughout the on- and off-transients. However, unlike moderate exercise, the [PCr] fall could be seen to continue throughout the exercise bout and did not come to a steady state (Fig. 2B and C). The response of the  $[\text{P}_i]$  peak was not consistent between subjects; however, in the example shown (Fig. 1B),  $[\text{P}_i]$  clearly 'decomposed' into two separate peaks during exercise (cf. Yoshida & Watari, 1994). This phenomenon



**Figure 1.** Example stack plots of the  $^{31}\text{P}$  spectra during moderate- (A) and high-intensity (B) rest-exercise-rest transitions

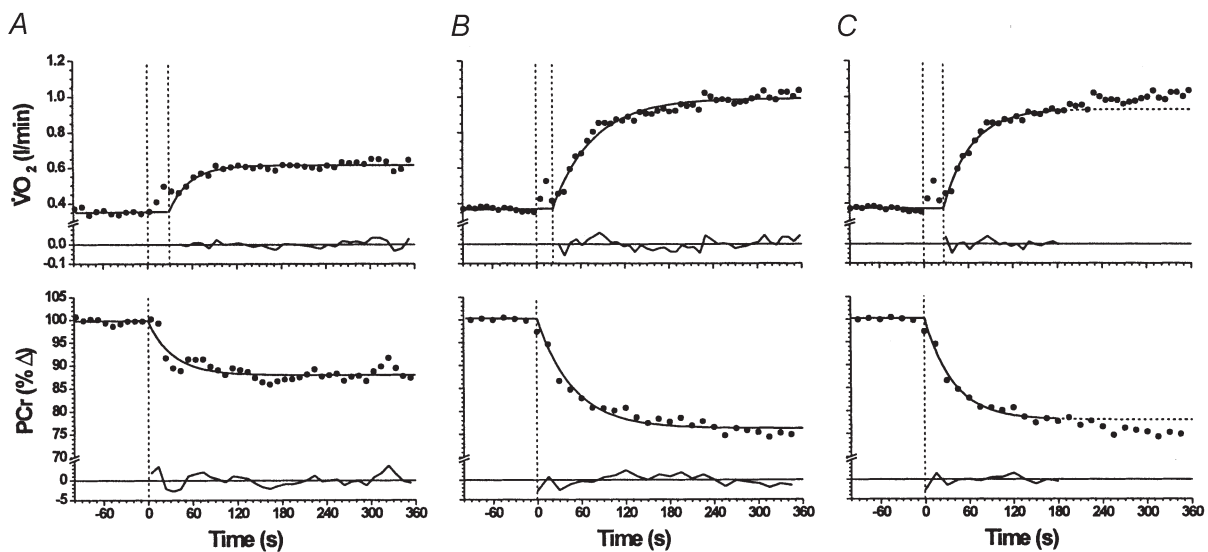
The stack plots are presented here in a reversal of the conventional format in order to visualise the dynamics of the  $[\text{P}_i]$  peak, which would otherwise be obscured by that of [PCr]. With this format and with the [PCr] peak used as the reference (0 p.p.m.), the [ATP] peaks appear positive and the  $[\text{P}_i]$  negative ( $\sim -5$  p.p.m.).

**Table 2. On- and off-transient PCr and  $\dot{V}_{O_2}$  amplitudes during moderate and high-intensity exercise**

Subject	Moderate					High				
	$\dot{W}$ (W)	$\Delta\dot{V}_{O_2,ss,on}$ (l min <sup>-1</sup> )	$\Delta\dot{V}_{O_2,ss,off}$ (l min <sup>-1</sup> )	$\Delta PCr_{ss,on}$ (%Δ)	$\Delta PCr_{ss,off}$ (%Δ)	$\dot{W}$ (W)	$\Delta\dot{V}_{O_2,ss,on}$ (l min <sup>-1</sup> )	$\Delta\dot{V}_{O_2,ss,off}$ (l min <sup>-1</sup> )	$\Delta PCr_{ss,on}$ (%Δ)	$\Delta PCr_{ss,off}$ (%Δ)
1	80	0.82	0.84	13.3	13.7	120	1.07	1.07	25.4	27.6
2	30	0.32	0.34	12.3	10.5	120	1.21	1.20	33.2	31.4
3	30	0.33	0.32	8.8	9.2	50	0.46	0.50	17.3	15.4
4	30	0.26	0.27	12.0	10.0	50	0.57	0.63	20.9	24.2
5	60	0.65	0.68	11.1	13.4	100	0.95	1.07	22.0	19.2
6	60	0.69	0.69	12.3	12.9	120	1.14	1.06	14.3	14.5
7	30	0.23	0.23	9.5	9.5	80	0.86	1.02	44.6	49.0
8	30	0.17	0.18	6.7	6.3	80	0.74	0.91	27.9	37.4
9	30	0.33	0.33	14.0	14.2	120	1.10	1.13	20.3	21.6
Mean	42	0.42	0.43	11.1	11.1	93	0.90	0.95	25.1	26.7
S.D.	19.2	0.2	0.2	2.3	2.6	29.6	0.3	0.2	9.2	11.2

**Table 3. Moderate-intensity time constants ( $\tau$ ) for on- and off-transient PCr and  $\dot{V}_{O_2}$**

Subject	On				Off			
	$\tau PCr$	$C_{95}$	$\tau \dot{V}_{O_2}$	$C_{95}$	$\tau PCr$	$C_{95}$	$\tau \dot{V}_{O_2}$	$C_{95}$
1	35	3.1	30	2.5	39	2.2	35	3.0
2	27	2.1	29	1.8	43	2.2	43	2.6
3	64	4.6	68	4.6	42	2.7	43	4.0
4	33	5.1	28	3.1	60	5.6	56	3.3
5	31	6.3	39	3.0	61	5.6	53	3.9
6	30	3.5	36	3.4	46	5.3	45	4.1
7	20	3.5	20	4.7	72	8.5	72	4.8
8	31	4.6	35	4.1	32	4.8	36	4.6
9	30	7.4	32	7.7	60	9.4	65	9.0
Mean	33	4.5	35	3.9	51	5.1	50	4.4
S.D.	12.2	1.6	13.5	1.7	13.1	2.6	12.7	1.9



**Figure 2.  $\dot{V}_{O_2}$  and [PCr] responses (for subject 2) with model fits and residuals**

A, moderate-intensity exercise; B, high-intensity exercise with the entire response modelled with a mono-exponential; C, high-intensity exercise with the mono-exponential fit limited to the fundamental phase, i.e. accounting for the early ‘cardiodynamic phase’ – see Methods for further elaboration.

**Table 4. High-intensity 'fundamental' component time constants ( $\tau$ ) for on- and off-transient PCr and  $\dot{V}_{O_2}$** 

Subject	On				Off			
	$\tau$ PCr	$C_{95}$	$\tau \dot{V}_{O_2}$	$C_{95}$	$\tau$ PCr	$C_{95}$	$\tau \dot{V}_{O_2}$	$C_{95}$
1	38	5.9	41	9.0	36	3.8	58	3.6
2	38	8.0	43	9.8	46	3.4	45	1.5
3	63	11.2	44	9.5	49	6.4	44	5.3
4	38	8.5	38	6.8	41	3.5	62	6.5
5	30	5.7	35	8.6	70	8.7	53	5.8
6	23	8.5	31	3.0	36	9.3	47	4.2
7	39	3.3	36	5.3	55	5.5	55	3.8
8	39	2.5	39	3.3	43	2.6	46	2.1
9	32	7.1	42	7.0	46	8.3	51	7.3
Mean	38	6.7	39	6.9	47	5.7	51	4.5
s.D.	10.9	2.7	4.2	2.6	10.6	2.6	6.3	1.9

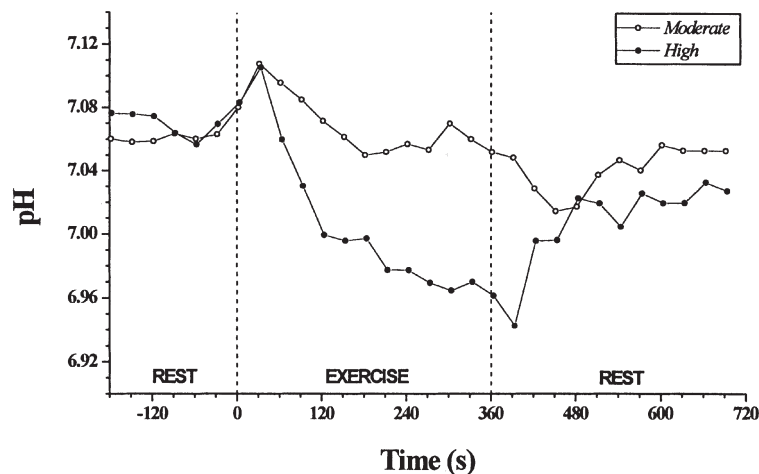
was apparent in four of the nine subjects during the high-intensity exercise and was not observed during moderate-intensity exercise in any subject.

As expected, the mono-exponential fit to the on-transient  $\dot{V}_{O_2}$  responses for high-intensity exercise was not adequate to characterise the response (Fig. 2B), and the fit was therefore limited to the fundamental exponential region (see Methods, Kinetic analysis). Interestingly, this was also the case for the on-transient of the [PCr] response (Fig. 2B), and thus the [PCr] responses were treated similarly to  $\dot{V}_{O_2}$ .

An example of resulting model fits to the fundamental component are shown in Fig. 2C, and the associated response amplitudes and time constants in Tables 2 and 4, respectively. The amplitudes for [PCr] and  $\dot{V}_{O_2}$  were larger than for the moderate-intensity exercise (Table 2): the fundamental  $\Delta[\text{PCr}]_{\text{ss}}$  averaged was  $25.1 \pm 9.2\%$ , and  $\Delta\dot{V}_{O_2, \text{ss}}$  was  $900 \pm 300 \text{ ml min}^{-1}$ . The approximate doubling

of the fundamental amplitude of  $\dot{V}_{O_2}$  between moderate- and high-intensity exercise was reflected in a similar ratio of the fundamental [PCr] decrement. The off-transient fundamental amplitudes averaged  $26.7 \pm 11.2\%$  and  $950 \pm 200 \text{ ml min}^{-1}$  for  $\Delta[\text{PCr}]_{\text{ss}}$  and  $\Delta\dot{V}_{O_2, \text{ss}}$ , respectively.

Limiting the analysis to the fundamental region of the responses produced average on-transient  $\tau$  values of  $38 \pm 11$  and  $39 \pm 4 \text{ s}$  for [PCr] and  $\dot{V}_{O_2}$ , respectively (Table 4), and while the shorter fitted region (average  $191 \pm 37$  and  $159 \pm 23 \text{ s}$  for [PCr] and  $\dot{V}_{O_2}$ , respectively) in the high-intensity exercise led to a larger confidence interval for parameter estimation, the  $C_{95}$  limits still remained within  $\pm 4 \text{ s}$  on average. The off-transient responses of both [PCr] and  $\dot{V}_{O_2}$  for high-intensity exercise were well characterised by a mono-exponential function (again with a delay arising from the early cardiodynamic region for  $\dot{V}_{O_2}$ ) in contrast to the corresponding on-transient responses at this intensity. This finding is consistent with those of the



**Figure 3. Intramuscular pH responses to moderate- and high-intensity constant load exercise in a subject without  $P_i$  peak splitting (subject 1)**

$\dot{V}_{O_2}$  responses to heavy-intensity exercise during cycle ergometry reported by Özyener *et al.* (2001). The off-transient fundamental  $\tau$  values averaged  $47 \pm 11$  and  $51 \pm 6$  s for [PCr] and  $\dot{V}_{O_2}$  respectively (Table 4). The associated confidence intervals were further improved, as essentially all of the entire 6 min transient was included in the fitting field ( $C_{95}$  averaged less than  $\pm 3$  s).

The final amplitude of [PCr] during the ‘slow component’ region of high-intensity exercise was, on average,  $13.9 \pm 9.1\%$  of the fundamental amplitude. The corresponding magnitude of the slow component of  $\dot{V}_{O_2}$  was  $15.3 \pm 5.4\%$ , thus illustrating that approximately 91% of the  $\dot{V}_{O_{2,sc}}$  magnitude was evident in an intramuscular measure of oxidative metabolism (Table 5). A Student’s paired *t* test did not reveal any significant difference between the magnitude of  $\dot{V}_{O_{2,sc}}$  and  $[PCr]_{sc}$ . However, the wide standard deviations illustrate that in some cases (e.g. subjects 1, 4, 8 and 9) almost the entire  $\dot{V}_{O_2}$  slow component was reflected intramuscularly, whereas in other cases as little as 17% (subject 6) of the  $\dot{V}_{O_2}$  slow component magnitude was manifest in the [PCr] profile.

**Intramuscular pH**

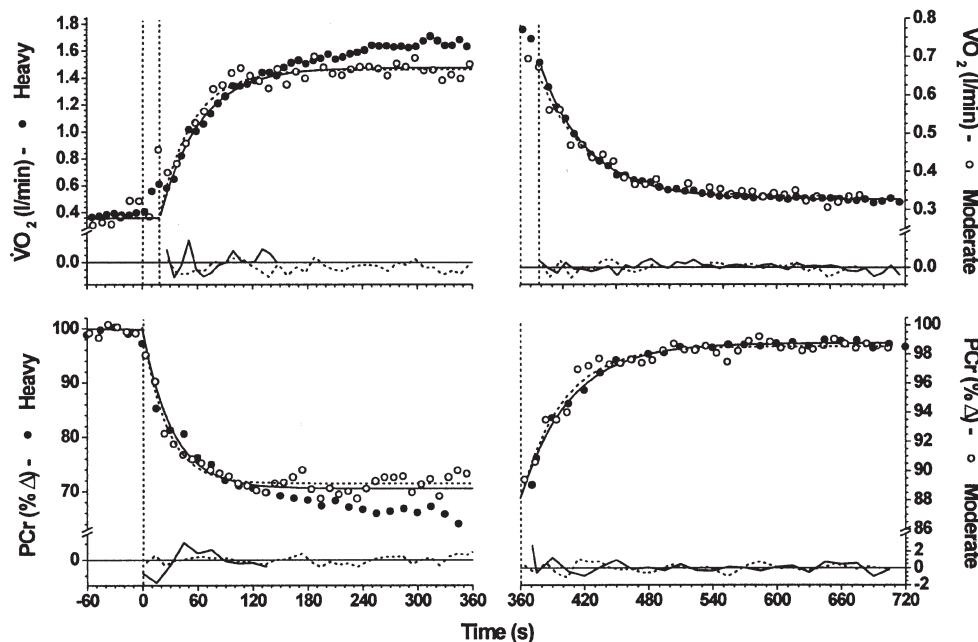
During moderate-intensity exercise intramuscular pH, as determined by frequency of the  $[P_i]$  peak relative to the [PCr] peak (eqn (1)), followed a similar characteristic time course in all subjects. An example (for subject 1) is shown in Fig. 3. Prior to exercise, the intramuscular pH averaged  $7.06 \pm 0.02$  for the nine subjects. At the onset of exercise

**Table 5. Magnitude of the slow components of [PCr] and  $\dot{V}_{O_2}$**

Subject	[PCr] <sub>sc</sub> (% phase II amplitude)	$\dot{V}_{O_{2,sc}}$ (% phase II amplitude)
1	8.2	5.8
2	5.7	16.4
3	8.3	10.9
4	13.9	9.1
5	12.4	17.2
6	2.9	16.8
7	17.9	19.6
8	28.7	20.4
9	27.1	21.2
Mean	13.9	15.3
SD	9.1	5.4

Calculation of  $[PCr]_{sc}$  and  $\dot{V}_{O_{2,sc}}$  is described in Methods.

there was typically a small rise in pH, peaking at approximately 30 s after exercise onset and averaging  $7.11 \pm 0.03$  (Fig. 3). The steady-state exercise pH value of  $7.05 \pm 0.05$  was not significantly different from rest, either pre- or post-exercise. pH fell immediately at exercise offset, reaching a nadir of  $6.98 \pm 0.07$  approximately 30 s later (Fig. 3). This nadir of 0.07 pH units below the resting value was similar in magnitude to the pH peak seen for the exercise on-transient, the mean of which was 0.05 pH units above resting.



**Figure 4. On-off asymmetry between the  $\dot{V}_{O_2}$  and [PCr] responses to moderate- (○, dashed lines) and high-intensity (●, continuous lines) exercise**

The responses have been normalised to the fundamental amplitudes to enable kinetic comparison of the responses at the two different intensities.

However, for high-intensity exercise, estimation of the pH was complicated by the non-consistent behaviour of the  $[P_i]$  peak at this intensity. For the four subjects who expressed a clearly discernible splitting of the  $[P_i]$  peak during this exercise, two distinct regional pH values could be estimated; these averaged  $6.62 \pm 0.13$  and  $7.05 \pm 0.04$  at end-exercise, compared with the pre-exercise baseline of  $7.06 \pm 0.02$  (Fig. 1B). Those subjects who did not clearly express a consistent splitting of the  $[P_i]$  peak during the exercise showed only a small degree of acidosis; i.e. an average decrement of  $0.1 \pm 0.03$  pH units at end-exercise (Fig. 3). As previously described by Bendahan *et al.* (1990), the off-transient pH dynamics in our study could not typically be distinguished, as the characteristic reduction of  $[P_i]$  to values below resting in this phase, and in some cases apparently zero, precluded estimation of the chemical shift.

### Cross-variable and cross-intensity comparisons

It is salient to note that during the exercise on-transients, the fundamental responses of both variables demonstrated similar average time constants of 33 and 35 s during moderate exercise, and of 38 and 39 s during high-intensity exercise for [PCr] and  $\dot{V}_{O_2}$ , respectively. Although this close kinetic coupling was maintained at the off-transient, the  $\tau$  values were typically greater than those at the on-transient (51 and 50 s during moderate exercise and 47 and 51 s during high-intensity exercise for [PCr] and  $\dot{V}_{O_2}$ , respectively). Repeated-measures ANOVA revealed that there was no effect of either intensity (i.e. moderate or high) or of variable (i.e. PCr or  $\dot{V}_{O_2}$ ) on the values of the fundamental  $\tau$ . However, a significant effect of transition (i.e. on- or off-transient) was established ( $P < 0.01$ ).

Also, the increment in the response amplitude above the fundamental, which has been previously well described for  $\dot{V}_{O_2}$  (i.e. the  $\dot{V}_{O_2}$  slow component), was shown to be evident in the profile of [PCr] (e.g. Fig. 2C). However, the off-transient responses did not express a discernible slow component in either variable. This is illustrated in Fig. 4, where the normalised and phase-aligned on- and off-transient  $\dot{V}_{O_2}$  and [PCr] responses of subject 2 from moderate- and high-intensity exercise may be compared. The close temporal relationships between the fundamental region of the on-transients of [PCr] and  $\dot{V}_{O_2}$  is clearly demonstrated at both exercise intensities. Furthermore, the slow components of both variables express a similar relative magnitude and time of onset. The off-transient responses, however, clearly did not differ between intensity: neither variable expressed a discernible influence of the on-transient slow component during high-intensity exercise. This inter-intensity dynamic linearity between the off-transients of  $\dot{V}_{O_2}$  and [PCr] responses was a typical and striking feature of the off-transient kinetics, with the  $\dot{V}_{O_2}$  and [PCr] kinetics from both intensities typically being indistinguishable.

## DISCUSSION

### Influence of exercise intensity on the $\dot{V}_{O_2}$ and [PCr] kinetics for the fundamental component

As type II fibres in skeletal muscle have been demonstrated to have up to two-thirds fewer mitochondria than type I fibres (Meyer *et al.* 1985), and also to manifest both a low oxidative efficiency and slow kinetics in response to square-wave exercise, the fundamental  $\dot{V}_{O_2}$  dynamics for high-intensity exercise might reasonably be expected to slow as the force contribution from type II fibres increases (Crow & Kushmerick, 1982; Barstow *et al.* 1996). Although there was a tendency for the fundamental responses of  $\dot{V}_{O_2}$  or [PCr] to be faster in moderate- compared with high-intensity exercise, this difference was not statistically significant. Our observations are consistent with the findings of Barstow & Molé (1991), Burnley *et al.* (2001) and of Özyener *et al.* (2001) for the fundamental  $\dot{V}_{O_2}$  kinetics during cycle ergometry. Furthermore, they add support to the suggestions of Barstow *et al.* (1990), Grassi *et al.* (1996) and Rossiter *et al.* (1999) that  $\dot{Q}_{O_2}$  and  $\dot{V}_{O_2}$  are not dissociated by more than 10%, in contrast to the suggestions of Cerretelli & di Prampero (1987), who considered the mean response time of the entire response rather than just the phase II component (for further discussion, see Rossiter *et al.* 1999).

While the proportion of fibre types contributing to force production during moderate- and high-intensity exercise in these studies is not known, it appears likely that the control of the fundamental component is independent of intensity over this range (consistent with previous suggestions by Barstow & Molé, 1991; Burnley *et al.* 2001; Özyener *et al.* 2001), and that the subsequent slow component of both  $\dot{V}_{O_2}$  and [PCr] corresponds to a supplemental influence exerted on the fundamental kinetics. Furthermore, the equality between  $\tau\dot{V}_{O_2}$  and  $\tau[\text{PCr}]$  that we have demonstrated for both intensities supports the notion that the fundamental  $\dot{V}_{O_2}$  kinetics are determined by an intramuscular mechanism(s) (Mahler, 1980; Hogan, 2001) rather than by muscle perfusion *per se*, as suggested by Hughson & Morrissey (1982), at least over the range of work rates investigated in this study. Our previous findings did not demonstrate such a striking identity between  $\tau\dot{V}_{O_2}$  and  $\tau[\text{PCr}]$  in every subject (Rossiter *et al.* 2001) during high-intensity exercise. It is possible that subject variability and/or exercise intensity may contribute to these differences.

### Dynamic asymmetry between on- and off-transient responses

In the present study, we have demonstrated a consistent 'on-off' asymmetry in the  $\dot{V}_{O_2}$  response kinetics for moderate-intensity exercise, with the off-transient  $\tau$  being longer than the on-transient (Table 3, Fig. 4). At first sight, this appears at odds with earlier reports of on-off symmetry for cycle ergometry (e.g. Linnarsson, 1974; Lamarra *et al.*



1983, 1987; Griffiths *et al.* 1986; Paterson & Whipp, 1991; Özyener *et al.* 2001). However, this may prove to be an overly simplistic description. For example, small but consistent deviations from dynamic linearity in the  $\dot{V}_{O_2}$  response to moderate-intensity cycling have been reported by Brittain *et al.* (2001) during square-wave transitions in different regions of the moderate-intensity domain (cf. Hughson & Morrissey, 1982); Fukuoka *et al.* (1997) during sinusoidal exercise; and Miyamoto & Niizeki (1992) for incremental and decremental ramps. Less is known about the dynamic linearity of [PCr] kinetics in this intensity domain: on-off [PCr] symmetry has been reported during electrically evoked exercise in rat muscle (Meyer, 1988); a small kinetic asymmetry was reported in isolated perfused dog muscle (Piiper *et al.* 1968; Piiper & Spiller, 1970).

Other potential influences on the symmetry of the on- and off-transient  $\dot{V}_{O_2}$  and [PCr] responses must not be discounted, however, for example: (1) an involvement of a component of obligatory anaerobiosis arising from the dynamics of cardiac output ( $\dot{Q}$ ) (Yoshida & Whipp, 1994); (2) asymmetries of intramuscular pH similar to those estimated here. However, these would be expected to be more marked at high- compared with moderate-intensity exercise; (3) the influence of a higher-order control system (Jeneson *et al.* 1996), or 'feedforward' control of  $\dot{Q}_{O_2}$  (Timmons *et al.* 1996); (4) influences of asymmetric  $[P_i]$  kinetics (e.g. Bendahan *et al.* 1990) to respiratory control mechanism(s) or (5) challenges to the underlying assumption that the constant-load work rate leads to a constant ATP requirement during the exercise period (Boska, 1994; Bangsbo *et al.* 2001; Rossiter *et al.* 2001; Giannesini *et al.* 2002). However, recently Kushmerick (1998) has clearly demonstrated the potential for kinetic asymmetry in the [PCr] response to exercise due to alterations in the creatine kinase (CK) equilibrium associated with changes in ATP utilisation that are close to those reported in the present results during knee-extensor exercise in humans (e.g. Table 3; Fig. 4). The mechanism(s) underlying the measured asymmetry in the present study, however, await(s) resolution.

### Inferences from the $\dot{V}_{O_2}$ and [PCr] slow components

The  $\dot{V}_{O_2,sc}$  has been shown to be a dynamic feature of  $\dot{V}_{O_2}$  that is superimposed onto the fundamental response to a square-wave work rate increase (Whipp & Mahler, 1980; Barstow & Molé, 1991; Paterson & Whipp, 1991; Poole *et al.* 1994; Özyener *et al.* 2001). This component of  $\dot{V}_{O_2}$  appears to be intimately related to the fatigue process, although its precise causes are poorly understood. By measuring blood flow and arterio-venous  $O_2$  content difference across an exercising limb, Poole *et al.* (1991) showed that the  $\dot{V}_{O_2}$  slow component (i.e. from 3 min after onset to the end of exercise) was significantly associated with limb  $\dot{Q}_{O_2}$ . These authors thus suggested that ~86 % of

the slow component of  $\dot{V}_{O_2}$  arose in the exercising muscle. Others (Billat *et al.* 1998; Özyener *et al.* 1999) have suggested a more crucial role of increases in unmeasured work done by the arms and other stabilising muscles during heavy-intensity cycling exercise. Furthermore, many other mechanisms have been suggested, such as increases in respiratory muscle work (Hagberg *et al.* 1978; Wasserman *et al.* 1995), increases in exercising muscle (or body) temperature via the  $Q_{10}$  effect causing up to an ~8 % increase in absolute  $\dot{V}_{O_2}$  with a 1 °C increase in muscle temperature, assuming a  $Q_{10}$  of 2.2 (Wilkie, 1968; Poole *et al.* 1988; Willis & Jackman, 1994; Koga *et al.* 1997), increases in interstitial potassium levels (Barnes, 1988; Medbo & Sejersted, 1990), and/or adrenaline and nor-adrenaline (Gollnick, 1978) stimulating metabolism. The slow component may also be due to alteration in CK activity rates (Kushmerick, 1998), possibly modulated by the ensuing acidosis associated with high-intensity exercise. However, the underlying mechanistic link(s) to  $\dot{V}_{O_2,sc}$  of such putative mediators have yet to be demonstrated. The present investigation has demonstrated that a similar slow component is evident in intramuscular [PCr] and the  $\dot{V}_{O_2}$  only at high-intensity work rates, where moderate-intensity exercise in the same subjects expresses first-order mono-exponential on-kinetics.

This demonstration of [PCr]<sub>sc</sub> is consistent with the suggestion by Conley *et al.* (2001) that [PCr] and pH interact via the CK reaction to 'set' [ADP] in contracting muscle. These authors have suggested that in order to stave off the effects of ensuing fatigue the muscle should 'keep [PCr] low and/or keep pH high'. This would have the effect of potentially maintaining [ADP], which, if it were the predominant feedback controller for oxidative phosphorylation, would avoid an inexorable rise in  $\dot{Q}_{O_2}$ . At work rates that lead to a metabolic acidosis, where pH is low or continually falling (as in high-intensity exercise in these studies), a reduction in [PCr] would be necessary in order to maintain ADP supply and therefore balance ATP supply to demand. However, it is interesting that this view is apparently not manifest during the fundamental phase of both  $\dot{V}_{O_2}$  and [PCr], as during this component of the responses the kinetics are manifest with an apparently invariant time course, i.e. moderate- and high-intensity  $\tau$  was the same in the on-transient fundamental phase, despite a falling pH during high-intensity exercise.

Our data suggest that the fundamental  $\dot{V}_{O_2}$  component gain is approximately constant for each individual (i.e.  $\Delta\dot{V}_{O_2,ss}/\Delta\dot{W}$  reflects the  $\Delta\dot{Q}_{O_2,ss}/\Delta\dot{W}$ ) and does not differ at different exercise intensities, consistent with the results of cycle ergometer exercise (e.g. Barstow & Molé, 1991; Paterson & Whipp, 1991; Özyener *et al.* 2001). However, the slow component appears to be superimposed upon this fundamental response, which causes a reduction in the oxygen utilisation efficiency of the muscular work (or an

increase in gain i.e.  $\Delta\dot{V}_{O_2,ss}/\Delta\dot{W}$  or  $\Delta[\text{PCr}]_{ss}/\Delta\dot{W}$ ). This slow-onset supplementary component is observable in the exercising muscle as an increase in the rate of [PCr] utilisation, reflecting a high phosphate cost of force production, rather than a high  $O_2$  cost of phosphate production. These current findings are in broad agreement with those of Poole *et al.* (1991) for  $\dot{V}_{O_2}$ . The data from our investigations, however, also show good dynamic association between the onset of the  $\dot{V}_{O_2,sc}$  and that of [PCr] (to within  $\sim 30$  s). Furthermore, as well as this close temporal association, the magnitude of the  $[\text{PCr}]_{sc}$  corresponds to 91 % on average of that for the  $\dot{V}_{O_2,sc}$  when normalised to the amplitude of the fundamental phase (despite a large standard deviation), the remainder of the response presumably residing in other mechanisms, such as intramuscular inefficiency of oxidative phosphorylation (as suggested by Meyer & Foley, 1996) or extra  $O_2$  cost from other sites.

From their investigations of the  $\dot{V}_{O_2}$  slow component in both running and cycling for groups of highly trained athletes familiar with both modalities (triathletes), Billat *et al.* (1998) have implicated additional unmeasured work from auxiliary muscles (e.g. in the arms) as contributing to the 'excess'  $\dot{V}_{O_2}$  during cycling, as the slow phase was markedly less prominent during treadmill exercise. However, the highly trained status of the subjects in the study of Billat *et al.* (1998) may be of some importance, as Williams *et al.* (2001) have recently measured a considerable  $\dot{V}_{O_2,sc}$  during treadmill running in 'less-elite' athletes.

In conclusion, we have demonstrated, firstly, that the time constants of the fundamental component for simultaneously determined [PCr] and  $\dot{V}_{O_2}$  dynamics cohere to within 10 %, during both the on- and off-transients to square-wave exercise of both moderate and high intensity. This is consistent with the notion that there is an apparent 'fundamental' phase of  $\dot{V}_{O_2}$ , the kinetics of which is independent of the energy requirement (i.e. the work rate imposed), as previously suggested (Barstow *et al.* 1996; Burnley *et al.* 2001; Özyener *et al.* 2001). Furthermore, it is possible that the dynamic asymmetry between the on- and off-transient responses in both [PCr] and  $\dot{V}_{O_2}$  at both intensities investigated could be a consequence of alterations in the CK equilibrium during the transition (cf. Kushmerick, 1998); however, why these asymmetries are not consistently demonstrated during cycle ergometry is, at present, unclear. Hence the dynamic responses of  $\dot{V}_{O_2}$  and [PCr] could retain linear first-order control, but via a transfer function that is dependent on CK equilibrium. Finally, our results demonstrate that  $\sim 90$  %, on average, of the magnitude of the  $\dot{V}_{O_2}$  slow component during high-intensity exercise is reflected within the exercising muscle by its [PCr] response, consistent with previous suggestions of the origin of the  $\dot{V}_{O_2}$  slow component (Poole *et al.* 1991),

and that the fundamental and slow component kinetics of  $\dot{V}_{O_2}$  reflect an intramuscular signal for oxidative metabolism.

## REFERENCES

- BANGSBO, J. (1996). Oxygen deficit: A measure of the anaerobic energy production during intense exercise? *Canadian Journal of Applied Physiology* **21**, 350–363.
- BANGSBO, J., KRUSTRUP, P., GONZALEZ-ALONSO, J. & SALTIN, B. (2001). ATP production and efficiency of human skeletal muscle during intense exercise: effect of previous exercise. *American Journal of Physiology – Endocrinology and Metabolism* **280**, E956–964.
- BARNES, W. S. (1988). Depressing effect of calcium antagonists on oxygen consumption in isolated skeletal muscle during potassium depolarisation. *Canadian Journal of Physiology and Pharmacology* **66**, 836–840.
- BARSTOW, T. J., BUCHTHAL, S., ZANCONATO, S. & COOPER, D. M. (1994). Muscle energetics and pulmonary oxygen uptake kinetics during moderate exercise. *Journal of Applied Physiology* **77**, 1742–1749.
- BARSTOW, T. J., JONES, A. M., NGUYEN, P. H. & CASABURI, R. (1996). Influence of muscle fiber type and pedal frequency on oxygen uptake kinetics of heavy exercise. *Journal of Applied Physiology* **81**.
- BARSTOW, T. J., LAMARRA, N. & WHIPP, B. J. (1990). Modulation of muscle and pulmonary  $O_2$  uptakes by circulatory dynamics during exercise. *Journal of Applied Physiology* **68**, 979–989.
- BARSTOW, T. J. & MOLÉ, P. A. (1991). Linear and non-linear characteristics of oxygen uptake kinetics during heavy exercise. *Journal of Applied Physiology* **71**, 2099–2106.
- BEAVER, W. L., WASSERMAN, K. & WHIPP, B. J. (1973). On-line computer analysis and breath-by-breath graphical display of exercise function tests. *Journal of Applied Physiology* **34**, 128–132.
- BENDAHAN, D., CONFORT-GOUNY, S., KOZAK-REISS, G. & COZZONE, P. J. (1990).  $P_i$  trapping in glycogenolytic pathway can explain transient  $P_i$  disappearance during recovery from muscular exercise. *FEBS Letters* **269**, 402–405.
- BILLAT, V. L., RICHARD, R., BINSSE, V. M., KORALSZTEIN, J. P. & HAOUZI, P. (1998). The  $\dot{V}_{O_2}$  slow component for severe exercise depends on type of exercise and is not correlated with time to fatigue. *Journal of Applied Physiology* **85**, 2118–2124.
- BOHNERT, B., WARD, S. A. & WHIPP, B. J. (1998). Effects of prior arm exercise on pulmonary gas exchange kinetics during high-intensity leg exercise in humans. *Journal of Applied Physiology* **83**, 557–570.
- BOSKA, M. (1994). ATP production rates as a function of force level in the human gastrocnemius/soleus using  $^{31}\text{P}$  MRS. *Magnetic Resonance in Medicine* **32**, 1–10.
- BRITTAIN, C. J., ROSSITER, H. B., KOWALCHUK, J. M. & WHIPP, B. J. (2001). The kinetics of  $O_2$  uptake in different regions of the moderate exercise intensity domain in humans. *Journal of Physiology* **531.P**, 44P.
- BURNLEY, M., DOUST, J. H., CARTER, H. & JONES, A. M. (2001). Effects of prior exercise and recovery duration on oxygen uptake kinetics during heavy exercise in humans. *Experimental Physiology* **86**, 417–425.
- CASABURI, R., WEISSMAN, M. L., HUNTSMAN, D. J., WHIPP, B. J. & WASSERMAN, K. (1979). Determinants of gas exchange kinetics during exercise in the dog. *Journal of Applied Physiology* **46**, 1054–1060.
- CERRETELLI, P. & DI PRAMPERO, P. E. (1987). Gas exchange in exercise. The Respiratory System. Gas Exchange. In *Handbook of Physiology*, section 3, *The Respiratory System*, vol. IV, *Gas Exchange*, chap. 16, pp. 297–340. American Physiological Society, Bethesda, MD, USA.

- CERRETELLI, P., SHINDELL, D., PENDERGAST, D. P., DI PRAMPERO, P. E. & RENNIE, D. W. (1977). Oxygen uptake transients at the onset and offset of arm and leg work. *Respiration Physiology* **30**, 81–97.
- CONLEY, K. E., KEMPER, W. F. & CROWTHER, G. J. (2001). Limits to sustainable muscle performance: interaction between glycolysis and oxidative phosphorylation. *Journal of Experimental Biology* **204**, 3189–3194.
- CROW, M. T. & KUSHMERICK, M. J. (1982). Chemical energetics of slow- and fast-twitch muscles of the mouse. *Journal of General Physiology* **79**, 147–166.
- FUKUOKA, Y., SHIGEMATSU, M., FUKUBA, Y., KOGA, S. & IKEGAMI, H. (1997). Dynamics of respiratory response to sinusoidal work load in humans. *International Journal of Sports Medicine* **18**, 264–269.
- GIANNESINI, B., IZQUIERDO, M., LE FUR, Y., COZZONE, P. J. & BENDAHAN, D. (2002). *In vivo* reduction in ATP cost of contraction is not related to fatigue level in stimulated rat gastrocnemius muscle. *Journal of Physiology* **536**, 905–915.
- GERBINO, A., WARD, S. A. & WHIPP, B. J. (1996). Effects of prior exercise on pulmonary gas-exchange kinetics during high-intensity exercise in humans. *Journal of Applied Physiology* **80**, 99–107.
- GOLLNICK, P. D. (1978). Delivery and uptake of substrates. In *Third International Symposium on Biochemistry and Exercise*, pp. 87–97, ed. LANDRY, F. & ORBAN, W. A. R., Symposia Specialists, Miami, FL, USA.
- GRASSI, B., POOLE, D. C., RICHARDSON, R. S., KNIGHT, D. R., ERICKSON, B. K. & WAGNER, P. D. (1996). Muscle  $O_2$  uptake kinetics in humans: implications for metabolic control. *Journal of Applied Physiology* **80**, 988–998.
- GRIFFITHS, T. L., HENSON, L. C. & WHIPP, B. J. (1986). Influence of peripheral chemoreceptors on the dynamics of the exercise hyperpnoea in man. *Journal of Physiology* **380**, 387–403.
- HAGBERG, J. M., MULLIN, J. P. & NAGLE, F. J. (1978). Oxygen consumption during constant-load exercise. *Journal of Applied Physiology* **45**, 381–384.
- HOGAN, M. C. (2001). Fall in intracellular  $P_{O_2}$  at the onset of contractions in *Xenopus* single skeletal muscle fibers. *Journal of Applied Physiology* **90**, 1871–1876.
- HOLLOSZY, J. O., OSCAI, L. B., MOLE, P. A. & DON, I. J. (1971). Biochemical adaptations to endurance exercise in skeletal muscle. In *Advances in Experimental Medicine and Biology*, vol. 11: Muscle Metabolism during Exercise, ed. PERNOW, B. & SALTIN, B., pp. 51–62. Plenum Press, London, UK.
- HUGHSON, R. L. & MORRISSEY, M. (1982). Delayed kinetics of respiratory gas exchange in the transition from prior exercise. *Journal of Applied Physiology* **52**, 921–929.
- HUNTSBERGER, D. V. (1961). *Elements of Statistical Inference*. Allyn and Bacon Inc., Boston, USA.
- JENESON, J. A. L., WISEMAN, R. W., WESTERHOFF, H. V. & KUSHMERICK, M. J. (1996). The signal transduction function for oxidative phosphorylation is at least second order in ADP. *Journal of Biological Chemistry* **271**, 27995–27998.
- JUBRAIS, S. A., ESSELMAN, P. C., PRICE, L. B., CRESS, M. E. & CONLEY, K. E. (2001). Large energetic adaptations of elderly muscle to resistance and endurance training. *Journal of Applied Physiology* **90**, 1663–1670.
- KOGA, S., SHIOJIRI, T., KONDA, N. & BARSTOW, T. J. (1997). Effect of increased muscle temperature on oxygen uptake kinetics during exercise. *Journal of Applied Physiology* **83**, 1333–1338.
- KROGH, A. & LINDHARD, J. (1913). The regulation of respiration and circulation during the initial stages of muscular work. *Journal of Physiology* **47**, 112–136.
- KUSHMERICK, M. J. (1998). Energy balance in muscle activity: Simulations of ATPase coupled to oxidative phosphorylation and to creatine kinase. *Comparative Biochemistry and Physiology B* **120**, 109–123.
- KUSHMERICK, M. J., MEYER, R. A. & BROWN, T. R. (1992). Regulation of oxygen consumption in fast- and slow-twitch muscle. *American Journal of Physiology* **263**, C598–606.
- LAMARRA, N., WHIPP, B. J., BLEMENBERG, M. & WASSERMAN, K. (1983). Model-order estimation of cardiorespiratory dynamics during moderate exercise. In *Modelling and Control of Breathing*, ed. WHIPP, B. J. & WIBERG, D. M. Elsevier Biomedical, Oxford, UK.
- LAMARRA, N., WHIPP, B. J., WARD, S. A. & WASSERMAN, K. (1987). Effect of interbreath fluctuations on characterizing exercise gas exchange kinetics. *Journal of Applied Physiology* **62**, 2003–2012.
- LANGSETMO, I. & POOLE, D. C. (1999).  $\dot{V}_{O_2}$  recovery in the horse following moderate, heavy and severe exercise. *Journal of Applied Physiology* **86**, 1170–1177.
- LINNARSSON, D. (1974). Dynamics of pulmonary gas exchange and heart rate changes at the start and end of exercise. *Acta Physiologica Scandinavica*, suppl., **415**, 1–68.
- MACDONALD, M., PEDERSEN, P. K. & HUGHSON, R. L. (1997). Acceleration of  $O_2$  kinetics in submaximal heavy exercise by hyperoxia and prior high-intensity exercise. *Journal of Applied Physiology* **83**, 1318–1325.
- MAHLER, M. (1980). Kinetics and control of oxygen consumption in skeletal muscle. In *Exercise Bioenergetics and Gas Exchange*, ed. CERRETELLI, P. & WHIPP, B. J., pp. 53–66. Elsevier/North-Holland Biomedical Press, Amsterdam, The Netherlands.
- MEDBO, J. I. & SEJERSTED, O. M. (1990). Plasma potassium changes with high-intensity exercise. *Journal of Physiology* **421**, 105–122.
- MEYER, R. A. (1988). A linear model of muscle respiration explains mono-exponential phosphocreatine changes. *American Journal of Physiology* **254**, C548–553.
- MEYER, R. A., BROWN, T. R. & KUSHMERICK, M. J. (1985). Phosphorus nuclear magnetic resonance of fast- and slow-twitch muscle. *American Journal of Physiology* **248**, C279–287.
- MEYER, R. A. & FOLEY, J. M. (1996). Cellular processes integrating the metabolic response to exercise. In *Handbook of Physiology*, section 12, *Exercise: Regulation and Integration of Multiple Systems*, ed. ROWELL, L. B. & SHEPHERD, J. T., chap. 18, pp. 841–869. American Physiological Society, Bethesda, MD, USA.
- MILSUM, J. H. (1966). Transient response characteristics. *Biological Control System Analysis*, pp. 115–138. McGraw Hill, New York.
- MIYAMOTO, Y. & NIIZEKI, K. (1992). Dynamics of ventilation, circulation and gas exchange to incremental and decremental ramp exercise. *Journal of Applied Physiology* **72**, 2244–2254.
- MOON, R. B. & RICHARDS, J. H. (1973). Determination of intracellular pH by  $^{31}\text{P}$  magnetic resonance. *Journal of Biological Chemistry* **248**, 7276–7278.
- ÖZYENER, F., ROSSITER, H. B., WARD, S. A. & WHIPP, B. J. (2001). Influence of exercise intensity on the on- and off-transient kinetics of pulmonary oxygen uptake in humans. *Journal of Physiology* **533**, 891–902.
- ÖZYENER, F., WARD, S. A. & WHIPP, B. J. (1999). Contribution of arm muscle oxygenation to the ‘slow component’ of pulmonary oxygen uptake during leg-exercise cycle ergometry. *Journal of Physiology* **515.P**, 73P.
- PATERSON, D. H. & WHIPP, B. J. (1991). Asymmetries of oxygen uptake transients at the on- and offset of heavy exercise in humans. *Journal of Physiology* **443**, 575–586.
- PIPER, J., DI PRAMPERO, P. E. & CERRETELLI, P. (1968). Oxygen debt and high-energy phosphates in gastrocnemius muscle of the dog. *American Journal of Physiology* **215**, 523–531.

- PIPER, J. & SPILLER (1970). Repayment of O<sub>2</sub> debt and resynthesis of high-energy phosphates in gastrocnemius muscle of the dog. *Journal of Applied Physiology* **28**, 657–662.
- POOLE, D. C., BARSTOW, T. J., GAESSER, G. A., WILLIS, W. T. & WHIPP, B. J. (1994).  $\dot{V}_{O_2}$  slow component: physiological and functional significance. *Medicine and Science in Sports and Exercise* **26**, 1354–1358.
- POOLE, D. C., SCHAFFARTZIK, W., KNIGHT, D. R., DERION, T., KENNEDY, B., GUY, H. J., PREDILETTO, R. & WAGNER, P. D. (1991). Contribution of exercising legs to the slow component of oxygen uptake kinetics in humans. *Journal of Applied Physiology* **71**, 1245–1253.
- POOLE, D. C., WARD, S. A., GARDNER, G. W. & WHIPP, B. J. (1988). Metabolic and respiratory profile of the upper limit for prolonged exercise in man. *Ergonomics* **31**, 1265–1279.
- ROSSITER, H. B., WARD, S. A., DOYLE, V. L., HOWE, F. A., GRIFFITHS, J. R. & WHIPP, B. J. (1999). Inferences from pulmonary O<sub>2</sub> uptake with respect to intramuscular [PCr] kinetics during moderate exercise in humans. *Journal of Physiology* **518**, 921–932.
- ROSSITER, H. B., HOWE, F. A., WARD, S. A., KOWALCHUK, J. M., GRIFFITHS, J. R. & WHIPP, B. J. (2000). Inter-sample [PCr] fluctuations by <sup>31</sup>P-MRS and parameter estimation of metabolic responses to exercise in humans. *Journal of Physiology* **528**, 359–369.
- STUBBS, M., VAN DEN BOOGAART, A., BASHFORD, C. L., MIRANDA, P. M. C., RODRIGUES, L. M., HOWE, F. & GRIFFITHS, J. R. (1996). <sup>31</sup>P-Magnetic resonance spectroscopy studies of nucleated and non-nucleated erythrocytes; time domain data analysis (VARPRO) incorporating prior knowledge can give information on the binding of ADP. *Biochimica et Biophysica Acta* **1291**, 143–148.
- TIMMONS, J. A., POUCHER, S. M., CONSTANTIN-TEODOSIU, D., WORRALL, V., MACDONALD, I. A. & GREENHAFF, P. L. (1996). Increased acetyl group availability enhances contractile function of canine skeletal muscle during ischemia. *Journal of Clinical Investigation* **97**, 879–883.
- VAN DER VEEN, J. W. C., DE BEER, R., LUYTEN, P. R. & ORMNIDT, D. (1988). Accurate quantification of *in vivo* <sup>31</sup>P MRS signals using the variable projection method and prior knowledge. *Magnetic Resonance in Medicine* **6**, 92–98.
- WASSERMAN, K., STRINGER, W. W. & CASABURI, R. (1995). Is the slow component of  $\dot{V}_{O_2}$  a respiratory adaptation to anaerobiosis? *Advances in Experimental Medicine and Biology* **393**, 187–194.
- WHIPP, B. J. (1987). Dynamics of pulmonary gas exchange. *Circulation* **76** (suppl. VI), 18–28.
- WHIPP, B. J. & MAHLER, M. (1980). Dynamics of pulmonary gas exchange during exercise. In *Pulmonary Gas Exchange*, vol. II, *Organism and Environment*, ed. WEST, J. B., pp. 33–96. Academic Press Inc., NY.
- WHIPP, B. J. & ÖZYENER, F. (1998). The kinetics of exertional oxygen uptake: assumptions and inferences. *Medicina Dello Sport* **51**, 139–149.
- WHIPP, B. J., ROSSITER, H. B., WARD, S. A., AVERY, D., DOYLE, V. L., HOWE, F. A. & GRIFFITHS, J. R. (1999). Simultaneous determination of muscle <sup>31</sup>P-phosphate and O<sub>2</sub> uptake kinetics during whole-body NMR spectroscopy. *Journal of Applied Physiology* **86**, 742–747.
- WHIPP, B. J., WARD, S. A., LAMARRA, N., DAVIS, J. A. & WASSERMAN, K. (1982). Parameters of ventilatory and gas exchange dynamics during exercise. *Journal of Applied Physiology* **52**, 1506–1513.
- WHIPP, B. J. & WASSERMAN, K. (1972). Oxygen uptake kinetics for various intensities of constant load work. *Journal of Applied Physiology* **33**, 351–356.
- WILKIE, D. R. (1968). Tissues subserving movement and conduction. In *Principles of Human Physiology*, ed. DAVSON, H. & EGGLETON, M., pp. 778–893. Lea & Febiger, Philadelphia, USA.
- WILLIAMS, C. A., CARTER, H., JONES, A. M. & DOUST, J. H. (2001). Oxygen uptake kinetics during treadmill running in boys and men. *Journal of Applied Physiology* **90**, 1700–1706.
- WILLIS, W. T., & JACKMAN, M. R. (1994). Mitochondrial function during heavy exercise. *Medicine and Science in Sports and Exercise* **26**, 1347–1354.
- YOSHIDA, T. & WATARI, H. (1994). Exercise-induced splitting of the inorganic phosphate peak: investigation by time-resolved <sup>31</sup>P-nuclear magnetic resonance spectroscopy. *European Journal of Applied Physiology* **69**, 465–473.
- YOSHIDA, T. & WHIPP, B. J. (1994). Dynamic asymmetries of cardiac output transients in response to muscular exercise in man. *Journal of Physiology* **480**, 355–359.

### Acknowledgements

This work was supported by The Wellcome Trust, grant number 058420. H. B. R. is supported by The Wellcome Trust-International Prize Travelling Fellowship.

### Author's present address

H. B. Rossiter: Department of Medicine, Division of Physiology 0623A, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92092-0623, USA. Email: hrossiter@ucsd.edu