Muscle energetics and pulmonary oxygen uptake kinetics during moderate exercise

THOMAS J. BARSTOW, STEVEN BUCHTHAL, STEPHANIA ZANCONATO, AND DAN M. COOPER

Division of Respiratory and Critical Care Physiology and Medicine, Departments of Medicine and Pediatrics and of Radiology, Harbor-University of California at Los Angeles Medical Center, Torrance, California 90509

Barstow, Thomas J., Steven Buchthal, Stephania Zanconato, and Dan M. Cooper. Muscle energetics and pulmonary oxygen uptake kinetics during moderate exercise. *J. Appl. Physiol.* 77(4): 1742–1749, 1994.—The present study tested whether, during moderate exercise, *1*) the dynamic responses of ADP and changes in free energy of ATP hydrolysis (ΔG_{ATP}) were similar to those of phosphocreatine [PCr; as would be expected for a simple controller of muscle respiration ($\dot{Q}O_2$)] and *2*) the rise in pulmonary O_2 uptake ($\dot{V}O_2$) during cycle exercise would reflect the rise in muscle $\dot{Q}O_2$ indicated by the calf PCr kinetics. The responses of PCr, P_i, ADP, and ΔG_{ATP} were measured from the calf in five subjects during supine treadle exercise using ^{31}P-magnetic resonance spectroscopy and compared with those for $\dot{V}O_2$, measured breath by breath during upright cycle exercise. The time constants for ΔG_{ATP} [24.2 ± 14.2 (SE) s] were not significantly different from those for PCr (26.3 ± 17.3 s) and P_i (30.7 ± 22.5 s) ($P > 0.05$). The time constants for *phase 2* $\dot{V}O_2$ (29.9 ± 16.8 s) were also similar to those of PCr. In contrast, the dynamics of ADP were distorted from those of PCr due to dynamic changes in pH. These results are consistent with mechanisms of respiratory control that feature substrate control by PCr or thermodynamic control through changes in ΔG_{ATP}. However, these results are not consistent with substrate control by ADP in a simple fashion. Furthermore, the similarity of time constants for *phase 2* $\dot{V}O_2$ and muscle PCr suggests that *phase 2* $\dot{V}O_2$ kinetics reflect those of muscle $\dot{Q}O_2$ in healthy subjects during moderate exercise.

aerobic metabolism; respiratory control

WHEN MYOSIN adenosinetriphosphatase turnover increases with exercise, the need for aerobic ATP resynthesis [oxidative phosphorylation ($\dot{Q}O_2$)] rises concomitantly. It remains unclear, however, how this need for ATP resynthesis is quantitatively communicated to the mitochondria. Several alternative mechanisms or models have been proposed to describe the control of mitochondrial respiration, including substrate control by *1*) phosphocreatine [PCr; as creatine (Cr)] in a linear fashion (9, 14, 22), *2*) ADP in a hyperbolic (Michaelis-Menten) fashion (11, 24), or *3*) thermodynamic control through changes either in the adenylate charge (ATP/ADP; Ref. 39) or in the cytosolic free energy of ATP hydrolysis (ΔG_{ATP}; Refs. 23, 31). Generally, distinction between these mechanisms has been attempted from comparisons under steady-state conditions of the relationships between work rate and/or muscle $\dot{Q}O_2$ and each of the putative controllers. However, with this approach, the results were equivocal: experimental data were reported that either support or rule out each of the mechanisms (14, 18, 32, 33). One criticism of the work in isolated muscles, however, is that the cellular and tissue conditions under which these experiments were conducted may or may not realistically reflect the in vivo state. One purpose of this study, therefore, was to evaluate these models of respiratory control in human skeletal muscle using ^{31}P-nuclear magnetic resonance spectroscopy (^{31}P-MRS).

It was recently suggested that distinction among these mechanisms as to which is the most likely controller of $\dot{Q}O_2$ should be made under conditions in which pH is changed (13) and/or during the transition from one metabolic rate to another, i.e., during unsteady states of exercise (18, 24). pH varies most dramatically during heavy exercise, with its associated lactic acid production and accumulation in the muscle (14, 28). However, even for moderate exercise in which there is little or no sustained lactic acidosis, pH may not be constant during the dynamic adjustment to a new steady state (12). In the present study, we examined whether dynamic changes in pH after the onset of moderate exercise significantly altered the kinetics of adjustment of ADP and ΔG_{ATP} relative to those of PCr. The fundamental assumption in this approach, as made by Meyer (31), is that the associated muscle $\dot{Q}O_2$ would rise in an exponential fashion; this has been well documented in other studies (27, 34). Furthermore, we assumed that the kinetics of PCr would reflect those of $\dot{Q}O_2$ (27) irrespective of whether PCr was acting as a primary controller (9) or simply as a capacitance to buffer ATP levels (30). Recently, Kushmerick et al. (24) reported first-order kinetics for PCr breakdown and resynthesis in cat biceps muscle, whereas breakdown, but not resynthesis, was first order as well for soleus muscle. However, because of the relatively long response kinetics of their isolated muscle preparations (equivalent time constants of ~6–14 min), it is unclear whether these conclusions would be applicable to in vivo exercising human muscle, in which the time constants for adjustment of O_2 uptake ($\dot{V}O_2$) and/or PCr are usually much faster (20–30 s in normal subjects) (5, 20). In this study, we compared the dynamic responses of PCr, ADP, and ΔG_{ATP} after the onset of moderate exercise in human calf muscle using rapid acquisition of ^{31}P-MRS data (every 12 s).

A second purpose of this study was to test the prediction from computer simulations of Barstow and coworkers (3, 5) that during moderate upright cycle ergometer exercise [i.e., below the lactic acidosis threshold (LAT)] the rise in pulmonary $\dot{V}O_2$ during *phase 2* (i.e., starting ~15–20 s after the onset of exercise) would reflect closely the rise in contracting muscle $\dot{Q}O_2$. To test this hypothesis, albeit indirectly, we compared the time constant for the rise in $\dot{V}O_2$ during *phase 2* of moderate

cycle ergometer exercise with the time constant for changes in PCr obtained above for the calf exercise in the same subjects. This assumed that PCr kinetics could be used to indicate $\dot{Q}O_2$ kinetics under these conditions (i.e., moderate exercise with adequate O_2 supply).

METHODS

Subjects

Five healthy volunteers (4 males, 1 female) aged 34 ± 9 yr consented to participate after information regarding the project was explained to them. All subjects were healthy and free of known diseases, but one (*subject 5* in Table 1) was a current smoker. This subject refrained from smoking for ≥1 h before each exercise bout. The project was approved by the Human Subjects Committee at Harbor-University of California at Los Angeles (UCLA) Medical Center.

Exercise Protocols

Each subject performed similar exercise tests (incremental and constant work rate) both on an electrically braked cycle ergometer (Lanooy) and on a specially built treadle ergometer similar to that of Quistorff (35), which could be used in the whole body imaging scanner. For the incremental test on the cycle ergometer, the work rate was increased in a continuous fashion (ramp) from loadless pedaling until volitional fatigue. Pulmonary gas exchange [$\dot{V}O_2$, CO_2 production ($\dot{V}CO_2$)], minute ventilation, and heart rate were determined on a breath-by-breath basis throughout the initial rest, exercise, and recovery periods, as described below. From these responses, the peak $\dot{V}O_2$ and LAT were determined, the latter noninvasively from the V-slope method, where LAT is defined as the nonlinear breakpoint in $\dot{V}CO_2$ relative to the rise in $\dot{V}O_2$ (8). From these responses, a work rate was chosen for the cycle ergometer test that represented moderate exercise (80% of LAT).

For the incremental exercise treadle test in the scanner, plantar flexion was performed with the right foot at a frequency of 1/s. Work rate was incremented every 64 s (or 2 spectra, see below) by increasing the pressure within a pneumatic piston that applied resistance to the treadle pedal. Exercise was continued with increasing work rate until either the frequency of 1/s or the full range of motion could no longer be sustained. ^{31}P-MRS spectra were obtained continuously at rest, during exercise, and for 1–3 min in recovery, as described below. A work rate on the treadle ergometer was then selected that could be easily tolerated for 6 min, which represented ~60% on average of the peak work rate obtained during the incremental test. The results of the incremental test for the calf exercise have been reported elsewhere (2).

After ≥1 h of rest after the incremental tests, or on another day, each subject performed transitions from unloaded cycling (cycle ergometer) or rest (treadle ergometer) to the preselected work rate. During the cycle tests, pulmonary gas exchange and heart rate were followed breath by breath, whereas ^{31}P-MRS spectra were obtained every 12 s during the treadle exercise in the scanner. Three to four replicate transitions were performed by each subject on the cycle ergometer and then averaged (see below). The spectrum-to-spectrum noise in the ^{31}P-MRS data was less than that for the gas-exchange data, and the response kinetics of PCr, P_i, ADP, and ΔG_{ATP} were determined from a single transition.

Measurement of Breath-by-Breath Gas Exchange

Pulmonary gas exchange ($\dot{V}O_2$ and $\dot{V}CO_2$), minute ventilation, and heart rate were measured for each breath using a computer-based system. Expired volume was measured using an Alpha Technologies VMM-2 turbine, corrected for inertial characteristics, whereas gas fractions of O_2 and CO_2 were determined by a Perkin-Elmer MGA 1100 mass spectrometer from gas drawn continuously from the mouthpiece. $\dot{V}O_2$ and $\dot{V}CO_2$ were calculated as previously described, with correction for the transport delay from mouthpiece to sensor (7).

^{31}P-MRS

^{31}P spectra were obtained from a 10-cm receive-only surface coil placed over the belly of the right gastrocnemius. The leg was then placed through a linear head coil that was used for radio-frequency excitation and tuned to ^{31}P, and the patient was inserted into the magnet and positioned such that the surface coil was located in the center of the magnet. The position of the surface coil was confirmed from ^{1}H scout images in both the axial and sagittal orientations. Homogeneity of the magnetic field was optimized by shimming on the proton signal of the tissue water. After switching to ^{31}P, continuous sequential spectra were obtained every 12 s during 1–3 min of rest, during the exercise test, and in recovery. The flip angle was 90°, the spectral width was 2,000 Hz, repetition time was 1 s, and the number of free induction decays summed per spectrum was 12 for the constant work rate test (thus, each spectrum represented the average over 12 s).

Off-line spectral data were processed with 3 Hz of exponential line broadening before Fourier transformation. Nonlinear least-squares regression techniques (Picker) were used to calculate baseline and areas under the spectral peaks. Peaks were assumed to have a combination of Lorentzian and Gaussian characteristics. The areas under P_i, PCr, and β-ATP peaks were corrected for possible spin-lattice relaxation time saturation effects using the correction factors derived from Arnold et al. (1). Cellular pH was determined from changes in the chemical shift between the P_i and PCr peaks (38).

Data Analysis

Calculation of ADP and ΔG_{ATP}. To convert peak areas to concentrations, the β-ATP peak was assumed to represent ATP and was set at 8.2 mM (21). Concentrations of P_i and PCr could then be estimated as the product of the ratio of the areas to ATP (as P_i/β-ATP and PCr/β-ATP) and 8.2 mM. Total Cr (TCr) was assumed to be constant throughout the experiment and equal to 4.5 × β-ATP or 36.9 mM (16, 21). Cr was then obtained as the difference between TCr and PCr. ADP was calculated assuming equilibrium of the Cr kinase (CK) reaction as

$$[ADP] = \frac{(0.74)[ATP]([TCr] - [PCr])}{(1.66 \times 10^9)(10^{-pH_{obs}})[PCr]} \quad (1)$$

where the constant 0.74 is the estimated monovalent ion activity coefficient (24) that corrects for the fact that observed pH (pH_{obs}) is an activity, the terms in brackets are concentrations, and 1.66×10^9 is the equilibrium constant for CK. Free magnesium was assumed to be 1 mM and unchanging throughout each experiment (25); this was verified for each subject from a lack of change in the chemical shift of the β-ATP peak relative to that of PCr (17). ΔG_{ATP} was calculated as

$$\Delta G_{ATP} = \Delta G_o + RT \ln \frac{[ADP][P_i]}{[ATP]} + RT \ln [10^{-(pH_{obs}-7)}] \quad (2)$$

where R is gas constant, T is absolute temperature, standard free-energy change (ΔG_o) is assumed to be −32 kJ/mol at pH 7.0 (24), and the value of RT at 37°C is 2.58.

Determination of response kinetics. The breath-by-breath $\dot{V}O_2$ responses were interpolated to create evenly spaced data once per second, time aligned to the start of exercise, and aver-

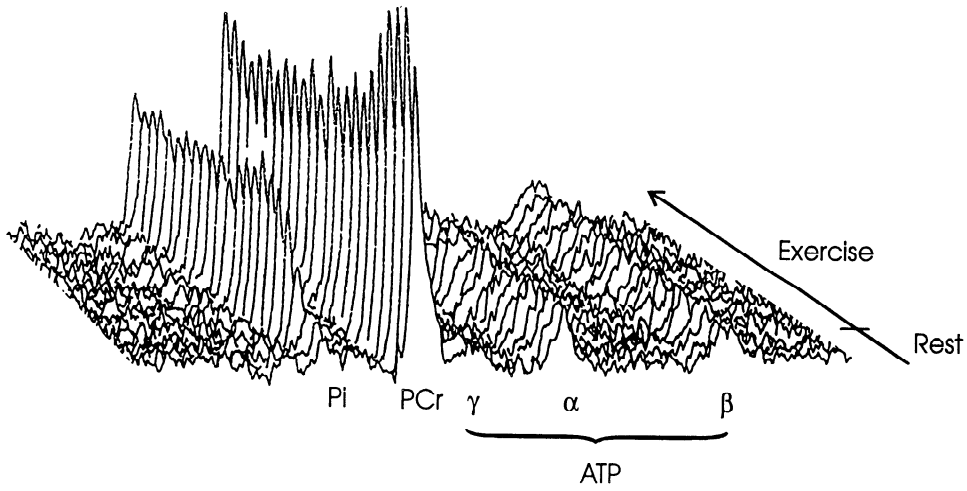

FIG. 1. Stacked plot of spectra for 1 subject, obtained every 12 s during transition from rest to moderate exercise. PCr, phosphocreatine.

aged across the transitions for each subject. Because the spectroscopy data were inherently evenly spaced, no initial processing was necessary. Data analysis involved fitting each variable of interest ($\dot{V}O_2$, PCr, P_i, ADP, and ΔG_{ATP}) to a monoexponential function using nonlinear regression

$$P(t) = P_b + A_1[1 - e^{-(t-Td)/\tau_1}] \quad (3)$$

where $P(t)$ is variable at time t, P_b is baseline (resting) value, A_1 is asymptotic or steady-state exercise value, τ_1 is time constant, and Td is time delay. For the fit of $\dot{V}O_2$ only, the 1st 15–20 s, representing the circulatory transport delay from muscle to lungs (*phase 1*; Ref. 3), were eliminated for this analysis.

Statistical Analysis

One-way analysis of variance for repeated measures was used to compare the similarity between time constants derived from *Eq. 1* for all of the variables, followed for significant results by Newman-Keuls multiple-range test to determine which variables were significantly different. Significance was declared at $P < 0.05$.

RESULTS

An example of the sequential spectra obtained for *subject 2* for constant work rate exercise on the calf ergometer is shown in Fig. 1. Figure 2 shows the mean responses of PCr, P_i, pH, estimated ADP, and ΔG_{ATP}, along with the best monoexponential fit (*Eq. 3*) to each mean response (except for pH) shown for illustration purposes. PCr fell and P_i and ΔG_{ATP} rose in curvilinear fashion toward the exercise steady-state levels, which were well described by exponential functions. Table 1 gives the individual time constants ± SE for the best fit to *Eq. 3*. The means ± SD for the time constants for PCr (26.3 ± 17.3 s), P_i (30.7 ± 22.5 s), and ΔG_{ATP} (24.2 ± 4.2 s) were similar ($P > 0.05$). pH showed a biphasic response, with an initial alkalinization beginning immediately after exercise onset, followed 24–48 s later by a mild acidification, which returned tissue pH to resting levels in three subjects and produced mild acidosis relative to rest in the other two subjects (pH = 6.85 and 6.92). In these two latter sub-

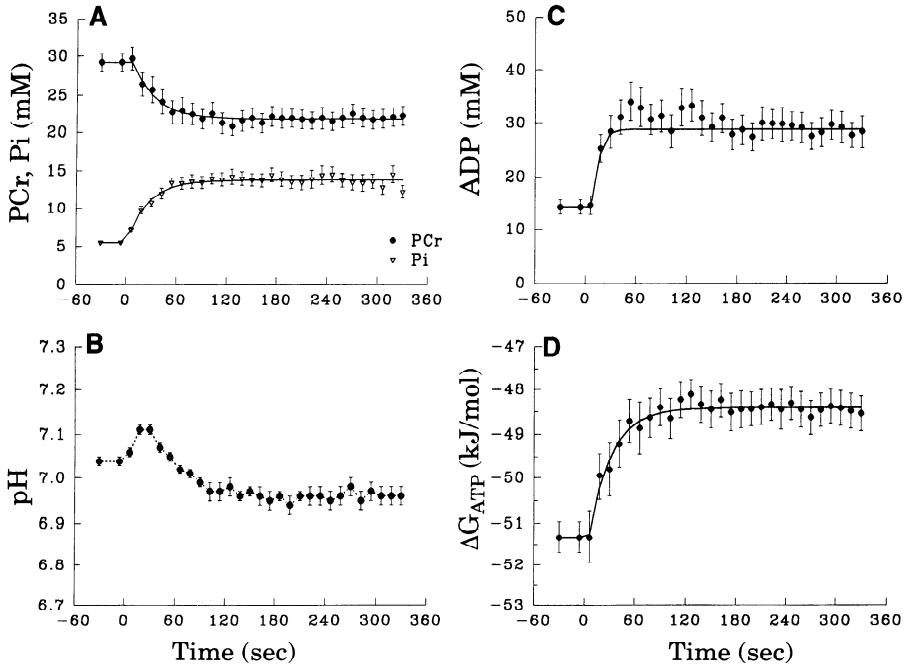

FIG. 2. Responses of PCr, P_i, pH, ADP, and changes in free energy of ATP hydrolysis (ΔG_{ATP}) during constant work rate calf ergometer exercise. Data are means ± SE. Best fit from *Eq. 1* for each mean response, except for pH, also shown.

TABLE 1. *Comparison of time constants for pulmonary $\dot{V}O_2$ and calf muscle phosphagens*

	τ, s				
Subject No.	$\dot{V}O_2$	PCr	P_i	ADP	ΔG_{ATP}
1	26.3±2.5	15.2±7.1	17.6±5.5	2.2±171.0	15.0±6.9
2	21.0±1.1	22.0±4.0	27.4±3.5	2.3±73.1	22.0±3.9
3	26.8±1.7	23.5±3.6	29.6±10.3	3.9±4.6	18.7±3.1
4	16.3±4.4	14.5±8.7	15.3±5.5	5.0±11.9	13.3±7.6
5	58.9±4.4	56.5±10.5	70.0±17.8	30.2±13.6	48.5±5.6
Mean	29.9±16.8	26.3±17.3	30.7±22.5	10.9±11.9*	24.2±14.2

Values are estimates ± SE; mean values are means ± SD. τ, Time constant; $\dot{V}O_2$, O_2 uptake; PCr, phosphocreatine; ΔG_{ATP}, changes in free energy of ATP hydrolysis. * Significantly different from $\dot{V}O_2$, PCr, P_i, and ΔG_{ATP} time constants, $P < 0.05$.

jects, the acidification was associated with a slight broadening of the P_i peak, but none showed evidence of splitting of the P_i peak.

ADP rose significantly more rapidly than PCr, P_i, or ΔG_{ATP} when described by an exponential function (time constant of 10.9 ± 11.9 s; $P < 0.05$). This discrepancy between ADP and PCr kinetics was due to dynamic changes in pH, as illustrated in Fig. 3. For the three subjects whose steady-state exercise pH was similar to rest (Fig. 3*A*), ADP rose to the steady-state level in a curvilinear manner that was well described by the exponential function. However, the kinetics were much faster than those predicted if pH were assumed constant at resting values (similar to the P_i/PCr ratio). In these three subjects, the speeding of ADP kinetics relative to those of PCr (and P_i) was the result of the early transient alkalinization, as might be intuited from *Eq. 1*. In the two subjects whose steady-state exercise pH was acidic relative to rest (Fig. 3*B*), the ADP response was biphasic, initially rising and then falling as pH fell below the resting level. In both of these subjects, the steady-state ADP level was less than the transient peak, with the actual level dependent on the degree of acidification: the more acidic, the lower the ADP. For the subject whose data are shown in Fig. 3*B*, the steady-state ADP was no different from the resting level. In both of these subjects, the dynamic response of ADP was obviously not well described by a monoexponential function (*Eq. 3*).

The mean response of all five subjects for the rise in $\dot{V}O_2$ during the cycle ergometer exercise is shown in Fig. 4, as well as a monoexponential fit (*Eq. 3*) to the mean data during *phase 2* for illustration. The time constants ± SE for the fits to the individual data for each subject are given in Table 1 and ranged from 16.3 to 58.9 s [29.9 ± 16.8 s (SD)]. The time constants for $\dot{V}O_2$ were not significantly different from those for PCr ($P > 0.05$).

The similarity of the time constants for $\dot{V}O_2$ and PCr across moderate work rates is shown in Fig. 5 for one of the subjects who completed transitions to more than one work rate on the calf ergometer and had previously completed transitions to several moderate work rates on the cycle ergometer for another study (10). Note both the remarkable constancy of the time constants independent of the work rate transitions for either ergometer and the similarity of the time constants for PCr and $\dot{V}O_2$ during *phase 2*.

DISCUSSION

Two important conclusions can be drawn from the present data. First, the findings that PCr and ΔG_{ATP}

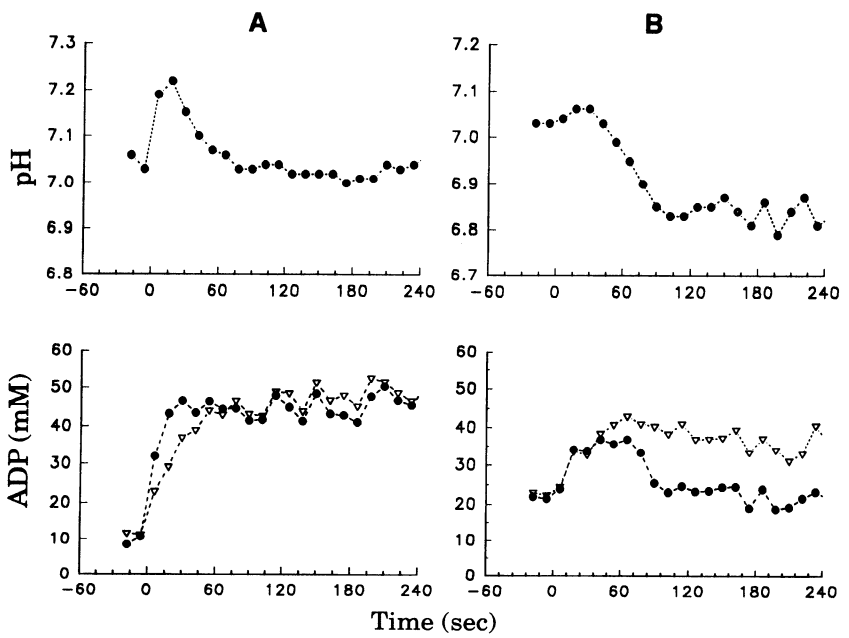

FIG. 3. Effects of changes in pH on ADP in 2 subjects. *Bottom*: ADP is corrected for changes in pH (●) and assumes pH to be constant at rest values similar to P_i/PCr ratio (▽). *A*: subject in whom there was no steady-state exercise acidification. Early alkalinization resulted in ADP kinetics that were much faster than those of PCr and P_i (as suggested by ▽). *B*: responses for another subject in whom there was slight steady-state acidification. This led to biphasic response of ADP, with steady-state exercise level similar to that of rest period.

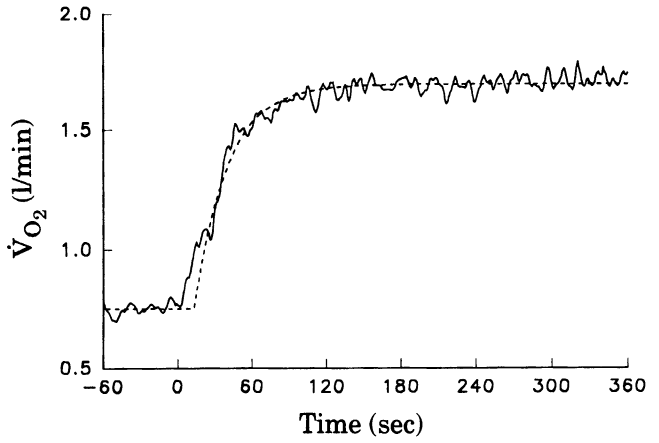

FIG. 4. Mean responses of 5 subjects for rise in O_2 uptake ($\dot{V}O_2$) during constant work rate cycle ergometer exercise. Dashed line, best fit, using *Eq. 1*, through mean data during *phase 2* (i.e., after 1st 15–20 s).

change in a simple and similar exponential fashion after the onset of exercise are consistent with one or both of these being primary controllers of skeletal muscle mitochondrial respiration during moderate exercise. Second, the similarity of the time constant for $\dot{V}O_2$ during *phase 2* with those of PCr and ΔG_{ATP} is consistent with our previous modeling predictions that *phase 2* $\dot{V}O_2$ kinetics would reflect muscle $\dot{Q}O_2$ kinetics (irrespective of the true controlling function).

Our first conclusion is based on the reasonable assumption that $\dot{Q}O_2$ under these conditions also exhibits first-order exponential behavior (18, 27, 34). Exponential behavior has previously been reported for PCr and ΔG_{ATP} in isolated muscles (18, 27, 31, 34) but only for PCr in contracting human skeletal muscle in vivo (20, 40). Furthermore, given the exponential responses of PCr and P_i, the observed dynamic changes in pH predict through the CK reaction (*Eq. 1*) that ADP cannot also change with the same exponential time course as PCr (and presumably $\dot{Q}O_2$). Although this does not rule out respiratory control by ADP, it does suggest that if ADP is the primary stimulator (controller) of mitochondrial respiration in contracting muscles, it must be acting dynamically through a complex function that is not first order. This appears true even for moderate exercise, in which there may be little steady-state change in pH from resting conditions.

Until recently, models of respiratory control in intact contracting skeletal muscle have focused primarily on substrate control. Differentiating between potential mechanisms of substrate control of respiration driven by PCr or ADP requires evaluation during conditions in which pH is altered (14), because when pH is constant, linear changes in PCr in the physiological range predict Michaelis-Menten (hyperbolic) changes in ADP (13). If we assume the CK reaction is at equilibrium (*Eq. 1*), the critical question addressed by this approach is whether ADP or Cr (from PCr) is the independent variable, with the other changing in a predictable fashion dictated by changes in pH. Evaluation of this question using steady-state data has shown variable results. Kushmerick et al. (24) found that steady-state $\dot{Q}O_2$ in contracting cat biceps and soleus muscles was related both linearly to PCr and P_i and hyperbolically to ADP across different work rates and pH responses. Similarly, Nioka et al. (33) found that when pH was altered during exercise in isolated rabbit gastrocnemius/soleus muscle groups by varying PCO_2 the tension-time integral (used as a surrogate for $\dot{Q}O_2$) was related to ADP along the same hyperbolic relationship irrespective of pH. However, their data revealed that PCr varied both with work rate and with pH in a nonlinear fashion. We (2, 41) and others (28) have previously shown that, in human mixed muscle performing incremental exercise, the onset of acidosis leads to nonlinear accelerated rates of breakdown of PCr and accumulation of P_i as work rate continues to increase. Although ADP in our data rose in a hyperbolic (Michaelis-Menten) fashion through the early work intensities and above the onset of significant acidosis up to ~70% peak work rate, it failed to continue to rise hyperbolically at the highest work rates. Thus, neither PCr nor ADP maintained response characteristics consistent with substrate control by either in a simple fashion as the sole determinant of respiratory control. Only ΔG_{ATP} changed in a consistent (linear) fashion throughout the range of aerobic work rates.

It has been suggested that, in addition to varying pH, differentiation between potential mechanisms for respi-

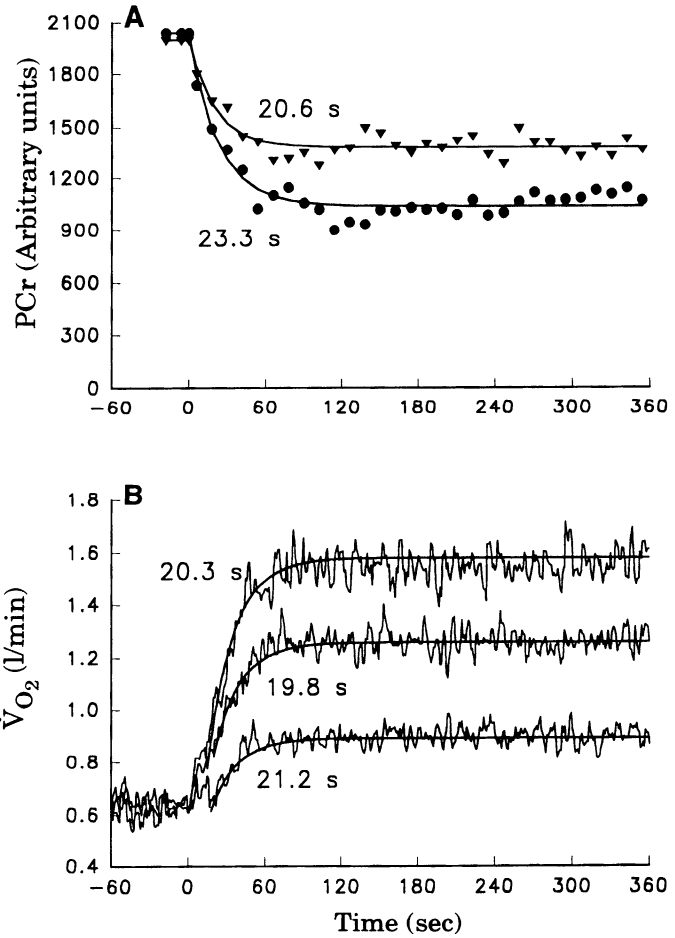

FIG. 5. Responses of PCr and $\dot{V}O_2$ for 1 subject who performed transitions to multiple work rates on calf (PCr) and cycle ($\dot{V}O_2$) ergometers. Note reproducible time constants both within and across exercise modalities for muscles of lower limbs performing moderate exercise.

ratory control in contracting muscles should be made from analysis of the kinetic changes in the putative controllers as metabolic rate is changed by changing work rate. Several early studies established both a theoretical and empirical connection between the kinetics of PCr and those of $\dot{Q}O_2$ (27, 31, 34). This led to the hypothesis that mitochondrial respiration was controlled by the rate and amount of Cr appearing at the mitochondrial CK (9, 22). However, Meyer recently showed that in Cr-depleted muscle the time constant for PCr breakdown was linearly related to TCr (29), as predicted if PCr acted simply as an energy buffer or capacitance (30), but the twitch force was not different from control muscles. This implied that diffusion of Cr to mitochondrial CK did not limit oxidative phosphorylation in normal muscle (32). In the study of Kushmerick et al. (24), analysis of the kinetics for PCr and P_i during recovery from stimulation revealed exponentiality for the biceps muscles but a transient overshoot in PCr and undershoot in P_i for the soleus. This led the authors to conclude that respiratory control in the biceps, but not in the soleus, could be explained by modulation of ADP at the mitochondria. In contrast, using computer simulations of an integrated model of respiratory control, Funk et al. (18) found that linear models based on PCr, P_i, or the phosphorylation potential [as $\log(ATP/ADP \times P_i)$, which is similar to ΔG_{ATP}] accurately predicted the time course of change in $\dot{Q}O_2$ of dog gracilis muscle. However, models that represented substrate control via either ADP alone or with ADP and P_i did not predict the observed data. In these studies, pH changed biphasically from 7.0 at rest to 6.5 during steady-state exercise. The data from the present study differ from those of Connett and co-workers (14, 18) in that pH only changed on average from 7.04 at rest to 6.95 during the steady state of exercise. Furthermore, the discrepancy here between the time constant for PCr and that for ADP was similar across the five subjects irrespective of the steady-state pH. Thus, our conclusions are similar to those of Connett and co-workers regarding the appropriateness of linear models of PCr and/or ΔG_{ATP} and the inappropriateness of a Michaelis-Menten model for ADP. However, our conclusions, in contrast to those of Connett and co-workers (14, 18), are based on much more subtle changes in pH that were observed during moderate exercise but that nonetheless led to measurable alterations in the dynamics of estimated ADP.

Taken collectively, the mechanism of respiratory control that seems most consistent with these previous observations and our present data would be one that incorporates thermodynamic control, expressed in our studies through changes in ΔG_{ATP}. As noted above, during incremental exercise in human gastrocnemius muscle, ΔG_{ATP} linearly rises from about -63 to -54 kJ/mol over the dynamic range of exercise (2). From the current data, ΔG_{ATP} approaches a new steady state exponentially. Thus, for both of these exercise forcing functions (incremental and square wave), ΔG_{ATP} follows the presumed response of muscle $\dot{Q}O_2$ more closely than PCr or ADP. Clearly, however, these data do not rule out other factors that could also modulate muscle respiration.

The similarity of PCr and $\dot{Q}O_2$ kinetics in isolated muscle is well established, as noted above. However, the relationship between $\dot{Q}O_2$ for the contracting muscles, which is difficult or impossible to measure in humans, and pulmonary $\dot{V}O_2$, which can be measured, is not clear. From previous computer simulations, we predicted that the kinetics of $\dot{V}O_2$ during *phase 2* of moderate cycle exercise (i.e., after the first 15–20 s) would reflect the time course for the rise in muscle $\dot{Q}O_2$ (3, 5). In the present study, we showed that the time constant for $\dot{V}O_2$ during *phase 2* of cycle ergometer exercise is very similar to that for PCr breakdown (and rise in ΔG_{ATP}) during calf exercise in the same subjects. Although this similarity is consistent with the model predictions and suggests a linkage between muscle PCr (and $\dot{Q}O_2$) and whole body $\dot{V}O_2$ kinetics, we recognize that this is not an ideal test of the prediction, since the muscles used during the cycle ergometer exercise include, but are not restricted to, the muscles of the calf group. Unfortunately, the $\dot{V}O_2$ signal from the contracting calf does not increase whole body $\dot{V}O_2$ sufficiently to describe the kinetics accurately.

Nonetheless, we believe that our conclusion is supported for the following reasons. First, the intensity of exercise was moderate, with little or no sustained lactate production, and the duration was relatively short (6 min) for both calf muscle and cycle ergometer exercise. Under these conditions it is reasonable to assume that the same type of muscle fibers (type I and maybe type IIa) were recruited for both exercise protocols. Although there are significant differences between time constants for $\dot{Q}O_2$ and PCr for different muscle fibers (especially I vs. IIb; Refs. 15, 24), a similarity in time constants exists across species for muscles containing predominantly oxidative fibers (types I and IIa; Refs. 15, 34). In addition, the kinetics of $\dot{Q}O_2$ and/or PCr are very stable across different work rate transitions within the same isolated muscle (31, 34). This is also observed in vivo for exercising human skeletal muscle, as seen in Fig. 5, where the time constants for PCr and $\dot{V}O_2$ are relatively constant across different moderate work rates and are similar to each other. Other factors that might conceivably alter the kinetics in the two protocols (i.e., differential sensitivity to circulatory O_2 delivery) would also be minimal for moderate exercise.

Another potential modulator of muscle $\dot{Q}O_2$ kinetics is the level of conditioning (19). In our study, the comparison of time constants in the present study was made for muscle groups not only in the same subjects but also in the same exercising limbs where the level of conditioning is likely to be similar. For these reasons, it seems reasonable to compare the kinetics of PCr in contracting calf muscle with the kinetics of pulmonary $\dot{V}O_2$ in the same subjects performing moderate cycle ergometer exercise.

The early alkalinization observed in the present study has been noted in isolated muscles at the onset of contractions (36) and most recently in femoral venous blood in exercising humans (37). It is likely due to the consumption of H^+ that accompanies the breakdown of PCr to P_i and Cr (26). This initial alkalinization would lead to bicarbonate retention in the muscle cell, which in turn would slow the release of CO_2 into the capillary and ve-

nous blood and subsequent appearance in the exhaled breath (4).

In conclusion, PCr, P_i, and ΔG_{ATP} all exhibit exponential behavior after the onset of constant work rate exercise. This response characteristic is consistent with cellular respiration being driven simply by adenosinetriphosphatase activity, in which control is expressed thermodynamically through changes in ΔG_{ATP}. Even for a moderate work rate transition, pH demonstrates a biphasic response. Early alkalinization, when measurable, led to kinetics for ADP that were much faster than the presumed rise in $\dot{Q}O_2$ (estimated from those of PCr), whereas any steady-state acidification relative to rest resulted in reduced steady-state ADP levels that could approach those of rest. These results are not consistent with a simple first-order model of respiratory control by ADP. Finally, the similarity between the time constants for PCr (and ΔG_{ATP}) and those of pulmonary $\dot{V}O_2$ during *phase 2* of moderate cycle ergometer exercise suggest that the *phase 2* $\dot{V}O_2$ kinetics are a good approximation of muscle $\dot{Q}O_2$ kinetics in healthy subjects under normal conditions.

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Address for reprint requests: T. J. Barstow, Div. of Respiratory and Critical Care Physiology and Medicine, St. John's Cardiovascular Research Center, Harbor-UCLA Medical Center, RB-2, PO Box 405, Torrance, CA 90509.

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