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Changes in potential controllers of human skeletal muscle respiration during incremental calf exercise

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Barstow, Thomas J., Steven D. Buchthal, Stefania Zanconato, and Dan M. Cooper. Changes in potential controllers of human skeletal muscle respiration during incremental calf exercise. *J. Appl. Physiol.* 77(5): 2169–2176, 1994.—The purpose of this study was to evaluate the consequences of nonlinear changes in phosphocreatine (PCr) and pH during incremental calf exercise on estimates of ADP and cytosolic free energy of ATP hydrolysis (ΔG_{ATP}). Six subjects performed incremental plantar flexion exercise on a treadle ergometer while muscle P_i metabolism (PCr, P_i, ATP) and pH were followed using ^{31}P-nuclear magnetic resonance spectroscopy. Changes in ADP and ΔG_{ATP} were estimated with the assumption that there was equilibrium of the creatine kinase reaction and homogeneous tissue metabolite pools. All six subjects showed a threshold for onset of cellular acidosis that occurred on average at 47.3 ± 12.7% of peak work rate (PWR). In five of the six subjects, PCr and P_i showed accelerated rates of change above the threshold for onset of cellular acidosis. In all six subjects, ADP, when correctly calculated considering changes in pH, rose in a curvilinear fashion that was well described by a Michaelis-Menten hyperbola through 60–100% of PWR, with a mean apparent Michaelis-Menten constant of 43.1 ± 17.1 μM ADP and a predicted maximal oxidative rate at PCr = 0, which was 241 ± 94% of PWR. ΔG_{ATP} rose linearly with work rate from −62.9 ± 1.8 kJ/mol during unloaded treadling to −55.0 ± 1.8 kJ/mol at PWR. If we assume a linear O_2 uptake-to-work rate relationship, these results are most consistent with control of respiration being exerted through ΔG_{ATP} under these conditions (incremental exercise by human calf muscle). These data suggest that the changes in PCr (and ultimately changes in ADP as well) with increasing work rate reflect shifts in substrate concentrations that are dictated and/or required under changing acid-base conditions by the linear rise in ΔG_{ATP}.

metabolic control; aerobic metabolism; oxidative phosphorylation

THE MECHANISM(S) underlying control of skeletal muscle respiration ($\dot{Q}O_2$) during exercise remains controversial despite intense investigation. Three major paradigms of respiratory control have been advanced: *1*) substrate control linearly by falling phosphocreatine (PCr) and implied linear rise in creatine (Cr) (4, 8), *2*) substrate control hyperbolically (Michaelis-Menten) by ADP (5, 6) or, alternatively, *3*) thermodynamic control exerted via changes in the cytosolic free energy of ATP hydrolysis (ΔG_{ATP}; Refs. 20, 37). As recently pointed out (8), when pH and the total P_i and adenylate pools are constant (conditions reflecting metabolic rates ranging from rest through moderate-intensity exercise), the paradigms are indistinguishable, i.e., a linear fall in PCr predicts a hyperbolic (Michaelis-Menten) rise in ADP and a linear rise in ΔG_{ATP}, when the Cr kinase (CK) reaction is assumed to be at equilibrium. Furthermore, under conditions of constant pH, the maximal theoretical oxidative rate of the Michaelis-Menten model (V_{max}) would be reached when PCr falls to zero. The slope of change in PCr to change in work rate or change in PCr to change in $\dot{Q}O_2$ for a given muscle reflects the sensitivity of respiratory control (9, 35) and appears to be proportional to mitochondrial density. Thus, muscles with higher mitochondrial content (red or type I and type IIa fibers) have a more shallow slope (greater increase in $\dot{Q}O_2$ for a given fall in PCr) than muscle fibers with lower mitochondrial content (white or type IIb fibers) (15).

Efforts to distinguish between models of respiration using acidotic conditions to alter the relationships between PCr, ADP, and ΔG_{ATP} have yielded conflicting results in animal experiments. For example, using the naturally occurring lactic acidosis that accompanies heavy exercise, Connett and Honig (8) found that measured muscle $\dot{Q}O_2$ was linearly related to both PCr and ΔG_{ATP} {as phosphorylation potential, ln ([ATP]/[ADP][P_i]), where terms in brackets are concentrations} but not to changes in ADP. However, Meyer (20) showed that PCr was not obligatory for tension development (and presumably $\dot{Q}O_2$), suggesting that either ADP or phosphorylation potential could be the relevant controller. Consistent with a model of control via ADP, Nioka et al. (24) found that hypercapnic-induced acidosis during moderate exercise did not alter the observed Michaelis-Menten relationship between ADP and work performed but did affect the relationship between O_2 uptake ($\dot{V}O_2$) and PCr. Finally, Kushmerick et al. (15) showed that, in cat biceps but not soleus, changes in respiration could be explained by Michaelis-Menten changes in ADP. In addition, both muscle types showed relatively linear relationships between $\dot{Q}O_2$ and PCr.

Resolving this controversy in human muscle has been difficult. Repeated muscle biopsy during exercise of both legs, where the muscle mass is great enough to estimate the muscle $\dot{Q}O_2$ from measurement of pulmonary $\dot{V}O_2$, is not routinely feasible. Conversely, utilizing the noninvasive technique of ^{31}P-nuclear magnetic resonance spectroscopy (MRS) has been limited generally to the study of relatively small muscle groups (forearm and calf) where the muscle $\dot{Q}O_2$ cannot easily be determined. Nonetheless, ^{31}P-MRS can be used to investigate how each of the putative determinants of respiratory control (PCr, ADP, and ΔG_{ATP}) respond during exercise. When ^{31}P-MRS has been utilized during incremental exercise in human skeletal muscle, two responses have been reported that have not been clearly described in animal muscle preparations: *1*) there is a threshold work rate or metabolic rate for cellular acidosis (pH_T) and *2*) above pH_T, the rates of change in PCr and P_i are accelerated

(18, 32). If there remains a tight coupling of $\dot{Q}O_2$ to PCr at these higher (acidotic) work rates, then the more rapid decline in PCr (and rise in Cr) would indicate a greater $\dot{Q}O_2$. In this case, one would predict that the greater $\dot{Q}O_2$ would also be accompanied by greater changes in ADP and/or ΔG_{ATP}. Alternatively, if ADP is the primary controller of respiration in skeletal muscle, then the growing acidification would require a greater fall in PCr levels and concomitant rise in Cr to achieve [ADP] necessary for the steady-state oxidative phosphorylation rate, as predicted by the CK reaction at equilibrium (*Eq. 1*). If this were the case, then correcting the estimates of ADP for changes in $[H^+]$ should lead to restoration of a Michaelis-Menten relationship between ADP and work rate or metabolic rate and a linear relationship between metabolic or work rate and ΔG_{ATP}. Finally, it was not intuitively obvious what the effects would be on the calculation of changes in ΔG_{ATP} of nonlinear kinetics of PCr and P_i and/or nonhyperbolic changes in ADP under acidotic conditions. The purpose of this study, therefore, was to investigate the relationship between PCr, ADP, and ΔG_{ATP} in human skeletal muscle during incremental exercise when changes in $[H^+]$ were accounted for.

METHODS

Subjects. Six healthy volunteers (5 males, 1 female) aged 34 ± 9 yr consented to participate after information regarding the project was explained to them. All were healthy and free of known diseases. The project was approved by the Institutional Review Board at Harbor-University of California at Los Angeles Medical Center.

Exercise protocol. Each subject performed progressive or incremental exercise to volitional fatigue in the supine position on a specially built treadle ergometer similar to that of Quistorff et al. (27). Exercise consisted of plantar flexion with the right foot at a frequency of 1/s. Work rate was incremented every 64 s (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 full range of motion could no longer be sustained.

^{31}P-MRS. ^{31}P-MRS studies were performed on a Picker model 1.5-T VISTA clinical spectrometer. Phosphorus 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 tuned to phosphorus for radio-frequency excitation, and the patient was inserted into the magnet and positioned such that the surface coil was located in the center of the magnetic field. 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, resting spectra were obtained with repetition times (TR) of 1 and 10 s to correct for possible differences in spin-lattice relaxation time (T_1; saturation effects). Continuous sequential spectra were then obtained every 32 s during 1–3 min of rest, during the exercise test, and in recovery. Flip angle was 90°, spectral width was 2,000 Hz, TR was 1 s, and the number of free induction decays summed per spectrum was 32. Thus, each spectrum represented the summed responses over 32 s.

Data analysis. Each data set consisted of 1,000 complex points per spectrum. 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 T_1 saturation effects by calculating the ratio of areas determined for each peak at TR of 10 s to the area determined for TR of 1 s. The ratios of correction factors for P_i to β-ATP (1.15) and PCr to β-ATP (1.31) were similar to those reported for fully relaxed spectra (TR = 20 s; Ref. 1). Cellular pH was determined from changes in the chemical shift between the P_i and PCr peaks (32).

Calculation of ADP and ΔG_{ATP}. To convert peak areas to concentrations, the β-ATP peak was assumed to represent total ATP and was set at 8.2 mM (11, 29). $[P_i]$ and [PCr] could then be estimated as the product of the ratio of the areas to ATP (as P_i to β-ATP and PCr to β-ATP) and 8.2 mM. Total Cr (TCr; [PCr] + $[P_i]$) was assumed to be constant throughout the experiment and equal to 4.5 × β-ATP, or 36.9 mM (9, 11, 29). Cr was then obtained as the difference between TCr and PCr. ADP was calculated with the assumption that equilibrium of the CK reaction is

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

where the constant 0.74 is the estimated monovalent ion activity coefficient (15) that corrects for the fact that pH_{ATP} is an activity, subscript obs indicates observed factors, and 1.66×10^9 is the equilibrium constant for CK. Free magnesium was assumed to be 1 mM and unchanging throughout each experiment (16). ΔG_{ATP} was calculated as

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

where G_o is Gibb's free energy, R is gas constant, and T is absolute temperature. ΔG_{ATP} is assumed to be −32 kJ/mol at pH 7.0 (15), and RT at 37°C is 2.58.

RESULTS

Changes in PCr, P_i, and pH during incremental exercise. Figure 1 shows a stacked plot of ^{31}P-MRS for one subject (*subject 1*), and the converted areas under the curves for PCr and P_i, and the calculated pH, are shown in Fig. 2. For *subject 1* and the other five subjects, PCr fell and P_i rose linearly during the early exercise levels, whereas pH was relatively constant at resting values (7.04 ± 0.02) until a particular metabolic rate or work rate was achieved (pH_T), above which pH fell significantly (end-exercise pH = 6.74 ± 0.09). pH_T occurred on average at 47.3 ± 12.7% of the peak work rate (PWR) or 33.2 ± 10.1% of the total PCr change (see Table 1). Above pH_T, both PCr and P_i showed accelerated rates of change (2.2 times the rate observed below pH_T) in five of the six subjects (*subjects 1–5* in Table 1). In the sixth subject, the breakpoint in pH occurred at a very light work rate (25% of PWR). In this subject, both PCr and P_i continued to change at their initial rates, which were closer to the accelerated rates observed in the other five subjects for the above-pH_T rates. For all six subjects, extrapolation of the initial slope of the fall in PCr to the intercept (PCr = 0) predicted a potential V_{max} that was 241% of the actual observed PWR (Table 2).

In four of the six subjects, PCr continued to fall linearly above pH_T until fatigue was encountered, as shown in Fig. 2. In two subjects (*subjects 3* and *4*), however, PCr and P_i reached maximum changes from rest values be-

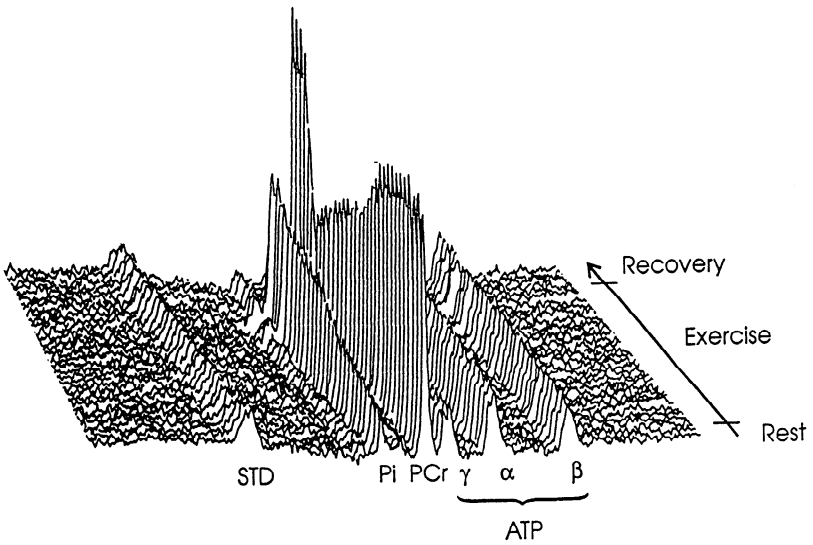

FIG. 1. Stacked plot showing ^{31}P-magnetic resonance spectra every 32 s for 1 subject (*subject 1*) during incremental exercise and recovery. STD, standard; PCr, phosphocreatine.

fore PWR was achieved. These levels were sustained for approximately two work rates. For these subjects, PWR and associated pH were defined as the values occurring when the peak changes in PCr and P_i were first observed.

Estimation of changes in ADP. Mean changes in metabolic rate (as %PWR) as a function of estimated ADP are shown in Fig. 3. Because PWR varied among subjects (Table 1), data were generated by interpolating the responses of each subject to increases in %PWR in increments of 0.1. Note the similarity of the two estimates until pH_T is exceeded, above which the correctly calculated ADP continues to rise hyperbolically but the uncorrected ADP rises very rapidly with further increases in work rate. In all subjects, ADP rose from 14.7 ± 8.9 μM at rest to 38.4 ± 20.8 μM at PWR. In four of the six subjects, the highest work rates (>60–75% of PWR; Table 2) were associated with little or no further change in ADP; in these subjects, the data up to this point only were fit. The rise in ADP over this range of 60–100% of PWR was well described by a Michaelis-Menten hyperbola in all six subjects. As there was no significant improvement in fit to the data by calculating the apparent V_{max} (as the asymptote of the ADP-%PWR relationship) compared with the assumption that V_{max} was equal to the value obtained by the linear extrapolation of PCr decline to PCr = 0 ($P > 0.05$ by F test for all 6 subjects), the latter fit was used for parameter determination. The resulting mean Michaelis-Menten constant for ADP was 43.1 ± 17.1 μM (Table 2).

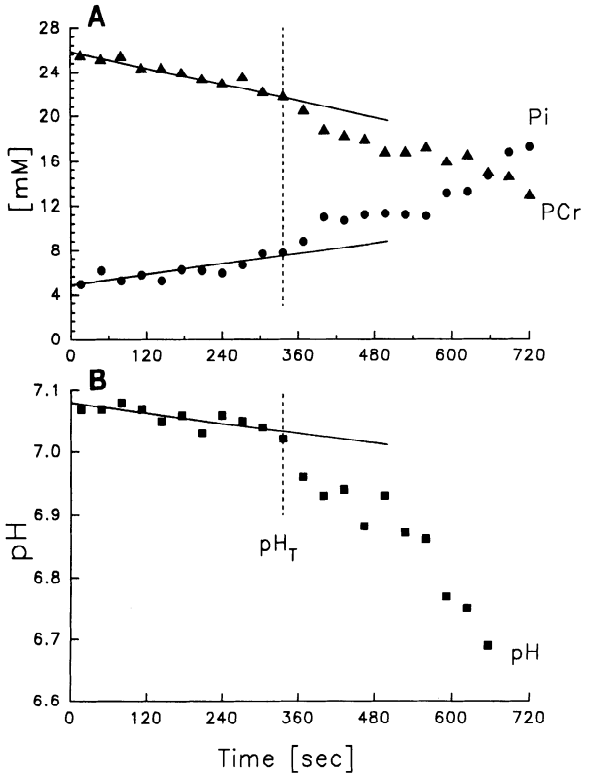

FIG. 2. Changes in PCr, P_i, and pH during incremental exercise shown for *subject 1*. pH_T, threshold for onset of cellular acidosis. PCr and P_i were converted to concentrations with assumption that ATP concentration (β-ATP peak in Fig. 1) was 8.2 mM. Note initial linear fall in PCr, rise in P_i, and relatively constant pH until pH_T, above which changes in PCr and P_i are accelerated as pH falls.

ΔG_{ATP}. Average ΔG_{ATP}'s are shown in Fig. 4. Interpolated data were created as for Fig. 3. As noted by others (15), correction for changes in pH had little effect compared with uncorrected estimates of ΔG_{ATP}. On average, ΔG_{ATP} rose linearly with work rate from −62.9 ± 1.8 kJ/mol at rest to −55.0 ± 1.8 kJ/mol at PWR.

β-ATP levels. In five of the six subjects, the area of the β-ATP peak was constant throughout the exercise period. In one subject (*subject 4*), however, β-ATP began declining at a work rate that was 73% of the PWR achieved (pH_T occurred at 36% of PWR; Table 1) and corresponded to the point at which PCr reached a minimum despite further increases in work rate. In this subject, the minimum β-ATP occurred 2–3 min into recovery and represented a 27% decline from rest and light exercise levels.

DISCUSSION

These results confirm the observations of Zanconato et al. (38), Marsh et al. (18), and Taylor et al. (32) regarding the occurrence of pH_T in human skeletal muscle during incremental exercise. Furthermore, these results show that when ADP and ΔG_{ATP} are correctly estimated considering these changes in pH, metabolic rate (esti-

TABLE 1. *End Ex, pH_T, and slopes for PCr*

Subject No.	PWR, psi	PCr End Ex, %rest	P_i End Ex, %rest	pH		pH_T		dPCr/dt	
				Rest	End Ex	%ΔPCr	%PWR	<pH_T, mM/min	>pH_T, mM/min
1	10	52.0	441	7.04	6.73	26.3	50.0	−1.17	−4.49
2	10	46.8	235	7.02	6.84	32.2	41.7	−1.29	−1.81
3	6	49.7	451	7.05	6.58	27.5	57.1	−1.62	−4.73
4	7	33.6	650	7.03	6.79	39.6	50.0	−2.85	−8.79
5	9	66.7	390	7.02	6.75	50.2	60.0	−1.00	−1.46
6	7	52.6	500	7.06	6.74	23.2	25.0	−3.67	−3.67
Means ± SD		46.4±7.5	445±136	7.04±0.02	6.74±0.09	33.2±10.1	47.3±12.7	−1.93±1.08	−4.16±2.64

End Ex, end-exercise values; pH_T, pH threshold; PCr, phosphocreatine; PWR, peak work rate; ΔPCr, change in PCr. dPCr/dt was estimated with assumption that ATP is 8.2 mM and total creatine (PCr + creatine) is 4.5 × total ATP (36.9 mM; see text for details).

mated as %PWR) follows changes in ADP as a Michaelis-Menten function and as a linear function of changes in ΔG_{ATP} over most or all of the functional metabolic range. These findings suggest that there are three regions of exercise intensity in the contracting muscles that parallel those described for two-legged cycle ergometer exercise (26, 36): *1*) moderate exercise, in which increases in metabolic rate are associated with a linear fall in PCr and rise in ΔG_{ATP} and a hyperbolic rise in ADP with little or no change in pH; *2*) heavy exercise, in which further increases in metabolic rate are associated with a falling pH, accelerated fall in PCr, but continued hyperbolic rise in ADP and linear rise in ΔG_{ATP}; and *3*) severe exercise, in which the fall in pH is more rapid, PCr continues to fall in an accelerated rate, ΔG_{ATP} continues to rise linearly, but ADP may not rise with the same relationship to work rate as that during moderate and heavy exercises.

The present results are most consistent with control of respiration being exerted in a linear nonequilibrium thermodynamic manner at the mitochondria (22). This conclusion is based on the observation of linear changes in ΔG_{ATP} throughout the range of work rates and tissue pH (Fig. 4). Similar results were reported by Connett and Honig (8) for a wide range of metabolic rates in isolated dog gracilis muscle. In the absence of changes in pH, ΔG_{ATP} is a predictable and linear function of PCr (and thus presumably $\dot{Q}O_2$ and work rate) over the physiologically functional range from 20 to 70% phosphorylation of the TCr pool (i.e., PCr/TCr; Ref. 19). Our data show, however, that under conditions of acidosis the relationship between changes in ΔG_{ATP} and changes in PCr is no longer linear. Because ΔG_{ATP} continues to change linearly as work rate increases, this would suggest that the breakdown of PCr passively accelerates in the presence of acidosis, as dictated by the conditions under which further linear changes in ΔG_{ATP} are achieved (i.e., *Eqs. 1* and *2*).

Our data would also appear to be consistent with substrate control of respiration by ADP up to 70–80% of PWR even under mildly acidotic conditions. Respiratory control by ADP has also been suggested by Kushmerick et al. (15) for cat biceps but not soleus muscles. In this model of respiratory control, the splitting of PCr might also be seen as a passive consequence of equilibrium of the CK reaction; PCr will fall with exercise to whatever level is necessary to produce the ADP level required to elicit the target respiratory rate at the mitochondrial translocase (*Eq. 1*; Ref. 13). Under acidotic conditions, the fall in PCr (and rise in Cr) would have to be greater for the same [ADP]. This is precisely what Nioka et al. (24) observed when pH was artificially lowered with hypercapnia in rabbit muscle performing moderate-intensity contractions. Thus, in both the nonequilibrium thermodynamic and the ADP substrate models, PCr functions primarily to buffer ATP levels and attenuate the rapid swings in respiratory rate that would otherwise be required during transitions to higher metabolic rates (19, 20, 23, 31).

Although our data could be used as evidence for ADP control of respiration in vivo, the results deviated from a hyperbolic relationship to work rate at the highest levels. This could be interpreted in at least two different ways. First, the data could imply that ADP is not the ultimate

TABLE 2. *Estimated change in ADP and ΔG_{ATP} during incremental calf exercise*

Subject No.	%PWR Used for ADP	ADP, μM			V_{max}, %PWR	ΔG_{ATP}			
		Rest	Peak	K_m		Rest	Peak, kJ/mol	Slope, $kJ \cdot mol^{-1} \cdot s^{-1}$	r
1	73	15.6	37.0	59.7	286	−62.3	−54.8	0.120	0.994
2	75	9.8	33.2	21.9	159	−60.7	−54.2	0.141	0.987
3	100	27.4	37.5	54.3	285	−62.5	−55.1	0.116	0.982
4	100	22.9	77.4	52.7	185	−61.5	−52.6	0.104	0.988
5	60	5.6	14.9	48.9	386	−64.9	−58.2	0.131	0.993
6	70	6.9	30.1	21.2	144	−65.2	−55.0	0.080	0.993
Means ± SD		14.7±8.9	38.4±20.8	43.1±17.1	241±94	−62.9±1.8	−55.0±1.8	0.115±0.022	

ΔG_{ATP}, change in Gibb's free energy of ATP hydrolysis; K_m, Michaelis-Menten constant; V_{max}, maximal oxidative rate of Michaelis-Menten model. V_{max} is predicted at PCr = 0. Range of data from rest to %PWR was used to calculate K_m for ADP and slope for ΔG_{ATP}.

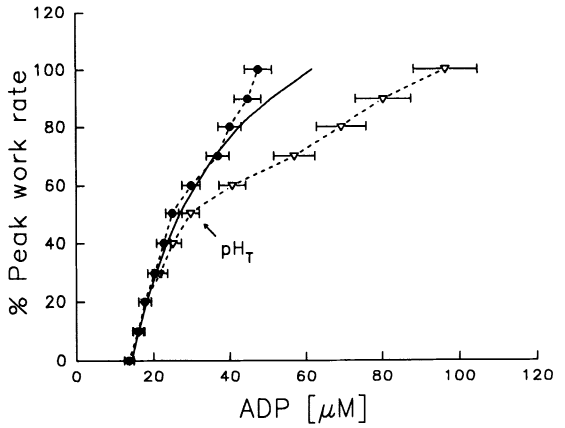

FIG. 3. Metabolic rate [as percentage of peak work rate (PWR)] as a function of estimated ADP. Group mean responses were obtained by interpolating each subject's responses to increments of 10% of PWR and then averaging across subjects. ▽, Estimated ADP with assumption of constant pH at rest for each subject (7.04); ●, ADP corrected for changes in (Δ) pH. Arrow denotes mean pH_T. Curvilinear rise of corrected ADP through 70% of PWR is well described by Michaelis-Menten hyperbola (Michaelis-Menten constant = 28.0 μM, predicted maximal oxidative rate of Michaelis-Menten model = 1.59 × observed PWR).

or primary stimulator of respiration in vivo as concluded by others (8, 22), since muscle $\dot{Q}O_2$ presumably continued to rise as work rate increased up to PWR and $\dot{Q}O_2$ for the muscle group. Alternatively, these data could suggest that ADP is controlling respiration up to ~70% of PWR but that above this relative metabolic rate additional factors are responsible for the further increase in $\dot{Q}O_2$. It is interesting to note that a threshold has been observed for bicycling exercise at a similar exercise intensity (26), which has been termed the fatigue threshold, or lower limit of sustainable power, because it represents the lower end of the hyperbolic (power output time to fatigue) relationship. Above this threshold, the time to fatigue falls as power output increases, with the product of the two constant. This threshold may represent a transition from a condition where ATP levels are buffered (rest through heavy exercise) to one where they begin to be depleted (7), as reflected by increased activity of adenylate kinase in an attempt to maintain ATP levels (33). With very heavy exercise, loss of total adenosine from the cellular pool is often observed, especially when the contracting muscle contains significant type IIb fibers. However, in this study, five of the subjects showed no measurable loss of adenosine, as reflected by a constant β-ATP peak throughout exercise and recovery. One subject did have a significant decrease in β-ATP at the highest work rates, which continued into recovery. The onset of the breakdown in β-ATP (67% of PWR) was at a metabolic rate above pH_T (50%). However, ADP continued to rise in a hyperbolic fashion and ΔG_{ATP} continued to rise linearly all the way to 100% of PWR.

In contrast to these models, if control of respiration is truly exerted at the mitochondria linearly by the declining PCr and concomitant rise in cytosolic Cr, as hypothesized by the PCr shuttle (4) and elsewhere (8), then the greater rate of breakdown of PCr under acidotic conditions, if still tightly coupled to oxidative phosphorylation, would predict that $\dot{Q}O_2$ also rises nonlinearly under acidotic conditions. Indirect evidence in support of this interpretation comes from the observation that during constant work rate cycle ergometry exercise above the lactic acidosis threshold there is a slowly developing additional component to the rise in pulmonary $\dot{V}O_2$ such that the steady-state or asymptotic $\dot{V}O_2$ is greater than that predicted from below lactic acidosis threshold work (3, 28, 35). However, this additional component to the whole body $\dot{V}O_2$ response is detectable only after the first 2–3 min of heavy constant work rate exercise and is usually not seen during incremental exercise of the duration seen here for the calf exercise (6–10 min). Under these conditions, $\dot{V}O_2$ rises linearly (10, 34) until peak $\dot{V}O_2$ is approached.

Although this additional component to the whole body $\dot{V}O_2$ response to heavy exercise likely originates within the exercising limbs (25), the precise site and mechanism(s) remain to be elucidated. Possible explanations for a nonlinear muscle $\dot{Q}O_2$ response during heavy constant work rate exercise (i.e., above pH_T) include nonlinear recruitment of motor units as work rate increases and/or recruitment of muscle fibers with greater change in PCr-to-change in $\dot{Q}O_2$ ratio. With respect to the latter, muscles with predominantly type II fibers (e.g., cat biceps brachii) show greater changes in PCr to achieve the same $\dot{Q}O_2$ as muscles with almost exclusively type I fibers (e.g., soleus; Refs. 14, 25). However, in these same studies, the slopes of the ADP-to-$\dot{Q}O_2$ and ΔG_{ATP}-to-$\dot{Q}O_2$ ratios were also greater for the cat biceps. Thus, if the greater PCr splitting observed in the present study during heavy or very heavy exercise reflected the recruitment of muscle fibers with less sensitive respiratory control (type IIb), one would have expected that during exercise above pH_T estimated ADP and ΔG_{ATP} would have also responded in a biphasic fashion, with greater changes in both for further increases in work or metabolic rate. However, both ADP and ΔG_{ATP}, when changes in pH were accounted for, appeared to change as continuous single functions (Figs. 3 and 4) at least through 60–75% of the range of tolerated work. Thus, our data do not support the concept of substrate control of respiration by PCr but rather a passive role to buffer [ATP] (31). This

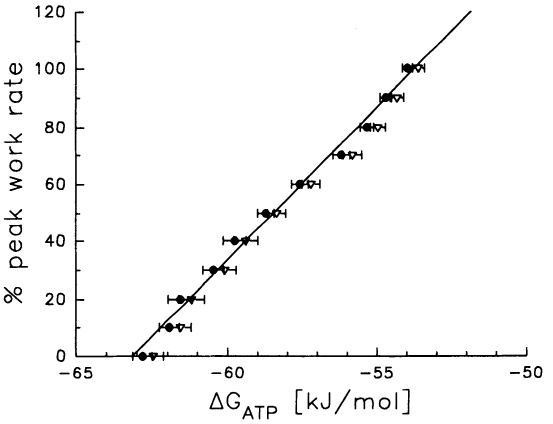

FIG. 4. Increased metabolic rate (as %PWR) as function of estimated rise in changes in free energy of ATP hydrolysis (ΔG_{ATP}). Mean response determined as described in Fig. 2. ▽, Data uncorrected for ΔpH; ●, data corrected for ΔpH. Regression equation is %PWR = 7.38 + 11.8 × ΔG_{ATP}, r = 0.997.

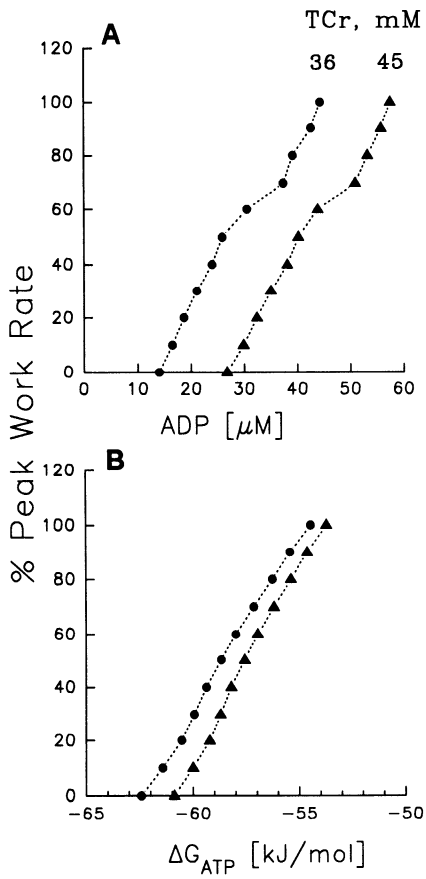

FIG. 5. Effects of different assumed values for total creatine (TCr) concentration on relationships between %PWR and ADP (*A*) and ΔG_{ATP} (*B*).

conclusion is similar to that of Meyer and Foley (22), which is based on work in Cr-depleted rats.

Calculations of ADP and ΔG_{ATP} require an estimation of the associated [Cr], which depends directly on the assumption of the average [TCr] in the muscles. Although [ATP] is well approximated as 8.2 mM in cell water across several species and muscle types (1), [TCr] is much more variable, with slow-twitch muscles and fibers having less TCr than fast-twitch muscles (21). Our assumption that TCr was 4.5 times ATP (9, 11) yielded a [TCr] of ~37 mM. Figure 5 shows the consequences on the mean data for estimated ADP and ΔG_{ATP} from Figs. 3 and 4 with the assumption that TCr is 5.5 × ATP or 45 mM (30). The primary consequence is a shift toward higher values of ADP and ΔG_{ATP} at any given %PWR. The apparent Michaelis-Menten constant for ADP increased from 43 to 56 μM, whereas there was little change in the slope of ΔG_{ATP}. This shift in the %PWR-ADP relationship emphasizes a significant dilemma in the substrate model for ADP and also for PCr, which is not present for the nonequilibrium thermodynamic model. The dilemma is that although the changes in ADP (and PCr) follow a given relationship as work rate is increased during exercise, this same relationship does not extend back beyond rest to project to zero [ADP] (and PCr equal to TCr) at zero metabolic rate. Rather, the exercise relationship(s) predicts positive [ADP] (and PCr < TCr) at a zero metabolic rate. The implication and conclusion of this observation is that the metabolic factors that determine the changes in PCr and ADP for a given increase in respiration rate with exercise may be different from those that determine this relationship at rest. Thus, neither the PCr nor the ADP substrate model completely explains the respiration rate from rest to presumably peak $\dot{Q}O_2$. However, the changes in ΔG_{ATP} are approximately linear throughout the range of metabolic rates and thus are not dictated by the concentration of any particular substrate of any particular reaction but rather reflect the status of the entire high-energy P_i storage system. For this reason in addition to those discussed above, it would appear that control of skeletal $\dot{Q}O_2$ by some indicator of high-energy P_i status in a nonequilibrium thermodynamic model, such as with ΔG_{ATP}, is most consistent with our data and the data of others (7, 8).

Our analyses and interpretation of the results of the present study are on the basis of two global assumptions: *1*) the metabolite changes as a function of work rate during the incremental exercise are similar to what they would be in a steady state and *2*) the concentrations can be spatially associated with each other [i.e., the contracting muscle(s) can be viewed as metabolically homogeneous]. Regarding the first assumption, responses of $\dot{Q}O_2$ in isolated muscle have been shown to behave as a linear system across a wide range of metabolic rates, tracking changes in work rate after a short delay that represents the time constant for the response (17). In a similar fashion, during short-duration incremental exercise such as that used here (6–10 min), whole body $\dot{V}O_2$ during cycle ergometer exercise in normal subjects rises linearly until peak $\dot{V}O_2$ is approached (10, 34). Thus, after the short time delay, in both isolated muscle and in whole body

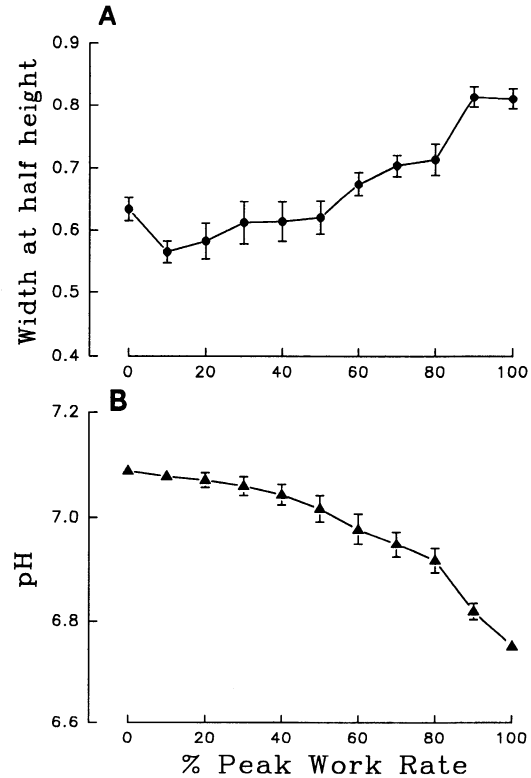

FIG. 6. Line width for P_i peak at one-half height (*A*) and pH (*B*) as functions of fraction of PWR. Line width reflects relative homogeneity of pH values within tissue being observed.

measurements, increases in $\dot{V}O_2$ occur linearly with increases in work rate during short-duration incremental exercise. This implies that the relationship between change in $\dot{V}O_2$ and change in work rate during incremental exercise testing is the same as would be seen, at least for the first few minutes, in constant work rate exercise. (However, as discussed above, whole body $\dot{V}O_2$ during constant heavy work rate exercise begins to exhibit nonlinear behavior after 2–3 min; Ref. 2.) In the present study, the relationship between instantaneous changes in metabolite concentrations and changes in work rate should therefore be comparable to those seen during at least the first few minutes of constant work rate exercise.

Regarding the relative spatial metabolic homogeneity of the contracting muscle, it is clear that there is potential for both macroheterogeneity (e.g., difference between fiber types; Ref. 15) and microheterogeneity (e.g., along the same capillary from arterial to venule end; for example, see Ref. 12). This concern exists for most or all of the probes currently used to examine intracellular events in vivo (including ^{31}P-MRS, needle biopsy, fluoroscopy, near-infrared spectroscopy, and other events). In the present study, [PCr], [P_i], [ATP], and [H^+] are assumed to be spatially homogeneous, especially and specifically at CK, so that ADP and ΔG_{ATP} could be calculated. In fact, the volume of tissue "observed" by the surface coil in this study reflects both the gastrocnemius and soleus muscles. Some anatomic heterogeneity may exist, therefore, because these muscles will likely differ in their fiber composition and thus in their TCr, mitochondrial density, and sensitivity of respiratory control. Further anatomic differences in fiber type distribution may be expected from intersubject variability. More to the point, however, slight functional heterogeneity could be detected at higher work rates, as shown in Fig. 6. Also shown is the mean pH response. As can be seen, the P_i line width broadens, implying a greater diversity of pH values within the muscles, above pH_T. However, although there may have been more diversity of pH in the contracting muscles, when metabolic homogeneity was assumed at CK, the patterns of change for both ADP and ΔG_{ATP} continued as they had during the more moderate work intensities during which pH was relatively constant. These observations are most consistent with a single primary metabolic compartment within the contracting muscle up to ≥70–80% of PWR.

In conclusion, PCr breakdown accelerates nonlinearly above pH_T during incremental exercise in human calf muscle(s). When corrected for the changes in cell pH, metabolic rate (as %PWR) rises in a Michaelis-Menten manner as a function of estimated ADP through 60–80% of PWR. However, the observation that %PWR rises linearly as a function of ΔG_{ATP} throughout the range of work rates is most consistent with control of mitochondrial respiration being exerted in a nonequilibrium thermodynamic manner through the phosphorylation potential or ΔG_{ATP}.

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