Free [ADP] and Aerobic Muscle Work Follow at Least Second Order Kinetics in Rat Gastrocnemius *in Vivo*

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Julie H. Cieslar and Geoffrey P. Dobson‡

From the Department of Physiology and Pharmacology, Schools of Biomolecular and Molecular Sciences, James Cook University, Townsville, Queensland 4811, Australia

The relationship between free cytosolic [ADP] (and [Pi]) and steady-state aerobic muscle work in rat gastrocnemius muscle *in vivo* using 31P NMR was investigated. Anesthetized rats were ventilated and placed in a custom-built cradle fitted with a force transducer that could be placed into a 7-tesla NMR magnet. Muscle work was induced by supramaximal sciatic nerve stimulation that activated all fibers. Muscles were stimulated at 0.1, 0.2, 0.3, 0.4, 0.5, 0.8, 1.0, and 2.0 Hz until twitch force, which could be placed into a 7-tesla NMR magnet. Muscle work does not obey Michaelis-Menten kinetics has recently resurfaced with the 31P NMR finding that the apparent kinetic order of [ADP] transduction is at least second order in intact human forearm (8). That free [ADP] and submaximal muscle work does not obey Michaelis-Menten kinetics has sparked a great deal of controversy (8, 33). The aim of our study was to determine the relationship between free cytosolic [ADP] (and [Pi]) and tension-time integral (TTI) in the rat gastrocnemius muscle *in vivo*. We show that free [ADP] (and [Pi]) and TTI followed an apparent second order function and that our data do not fit a Michaelis-Menten relationship.

EXPERIMENTAL PROCEDURES

Animal Preparation—Male Sprague-Dawley rats (350–400 g body weight) were obtained from Animal Resources Center, Canningvale, Western Australia, Australia. Animals were housed in the James Cook University small animal facility with free access to food and water. Animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (60 mg/kg), tracheotomized, and ventilated at the rate of 75 breaths/min with a tidal volume of ~25 ml to maintain arterial pH (7.4 ± 0.5), PCO2 (40 ± 5 mmHg), and PAO2 (90 ± 10 mmHg) (S.E.). Blood pH and gas tensions were analyzed using a Ciba-Corning 865 series analyzer. The right carotid artery was cannulated with heparinized (100 units/ml) polyethylene tubing connected to a Statham P23XL pressure transducer coupled to a MacLab for continuous measurement of arterial blood pressure. Anesthesia was maintained by delivering 1% isoflurane in compressed air via artificial ventilation at a rate of 0.5 liter/min. The entire surgical procedure was performed on a thermostatically regulated heating pad (37 °C).

Muscle Stimulation and Force Measurements—The right hindlimb was shaved, and two brass pins were driven through the calcaneum and

The abbreviations used are: TTI, tension-time integral; PCr, phosphocreatine; Cr, creatine.
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The muscle was adjusted to the length at which maximum twitch force was developed during a supramaximal contraction in response to a square-wave pulse (0.1 ms, 5–15 V). The TTI, defined as the area under the twitches over the acquisition period in the NMR spectra, was used as an index of muscle work with units of Newtons second (N s). The twitch tension-time integral was found to be a linear function of stimulation frequency during steady-state aerobic work (data not shown). The equation to the line was TTI = 46.98 stimulation frequency + 1.99 giving a TTI of 1.37 for the rate served as its own control precluding the need to normalize the data by muscle volume. The whole apparatus was introduced into the bore of an Oxford Instruments horizontal NMR magnet.

Nuclear Magnetic Resonance—31P NMR experiments were performed at 121.47 MHz in the 110-mm bore of an Oxford 7T superconducting magnet. A three-turn surface coil (14 mm outer diameter) was placed at the center of the gastrocnemius muscle and served as transmitter and receiver. A small latex balloon filled with a solution of 10 mM phenylphosphonic acid in saline was placed above the coil in the same geometrical arrangement as the muscle below and served as an external standard. Magnetic field homogeneity was optimized by observing the off-resonance proton signal of muscle water. The surface coil was tuned and matched to resonate at 121.47 MHz. All NMR experiments were performed using a radio frequency pulse of 10 μs duration, which was transmitted at a near 90° flip angle by the surface coil. 31P NMR spectra were collected as 9600 data points using a 20-s relaxation delay to the area of the spectra with a 1-s relaxation delay time of 1 s. The free induction decays were multiplied by a line broadening factor equivalent to 20 Hz to improve signal-to-noise ratio. Resultant line widths for the in vivo muscle experiments were typically 40–60 Hz. The radio frequency pulse was not synchronized with a nerve stimulus. The advantage of 31P NMR is that the method is noninvasive and provides continuous measurements of phosphorous metabolites in real time in one animal, including direct estimates of pH and free [Mg2+]i. The limitation of the NMR technique, as with conventional metabolic analysis, is that the measurements cannot discriminate the different contributions of fiber types.

Experimental Protocol and Definition of Steady State—Fully relaxed spectra with a relaxation delay of 20 s was collected for 30 min at the beginning of each experiment to determine the saturation correction factors for each of the phosphorus-containing compounds (see “Quantitation of NMR Spectra”). Control spectra were obtained for 7 min (256 transients) before the muscle was stimulated to contract isometrically at frequencies of 0.1, 0.2, 0.3, 0.4, and 0.5 Hz and either 0.8 Hz (n = 4), 1 Hz (n = 7), or 2 Hz (n = 7). For each stimulation frequency in the three groups, spectra were acquired in 4-min blocks each with 128 transients. Muscles were allowed to recover for 20 min between each stimulation frequency to restore the contraction force and PCr and P1 to prestimulation levels. The total time for each of the three protocols was about 3 h. The force and NMR data were then analyzed to establish a steady state in which twitch tension and PCr and P1 were constant in two consecutive spectra (9, 10). We showed that steady state was reached between 8 and 12 min of stimulation up to 0.8 Hz (see “Results”).

Parallel experiments were carried out on the laboratory bench and muscles were freeze-clamped following 10 min of stimulation at each of the frequencies using a nitrogen tongs in liquid nitrogen. The freeze-clamped muscles were ground to a fine powder under liquid nitrogen, the connective tissue was removed, and the powder kept at −80 °C until analysis of lactate, glycogen, pyruvate, PCr, and Cr. Conversion of total tissue contents (micromoles/g wet weight) into intracellular concentrations (micromoles/ml) were carried out using our published extracellular space values and total tissue water contents reported for rat gastrocnemius in vivo (11). In the present study we also measured the extracellular spaces at 1 and 2 Hz and found them to be 18% and 17%, respectively. The values were not significantly different from the 16% we report in vivo (11).

Biochemical Assays—All chemicals used in the metabolic assays were purchased from either Sigma or Roche Molecular Biochemicals and were of the highest grade. Frozen tissue (~100 mg) was homogenized in equal volumes of ice-cold 0.1 M HCl in methanol and 3.6% perchloric acid (12) using glass beads in a high speed Biospec mini-BeadBeverge. The homogenate was centrifuged (9,000 × g; 2 min) and a volume of acid-extract removed and mixed with an aliquot of KHCO3 (0.3 and 0.2 M stocks) to neutralize (pH 6–7). The supernatant was immediately measured either spectrophotometrically or fluorometrically for pyruvate, lactate, PCr, and creatine according to the methods of Passoneau and Lowry (12). Total tissue glycogen was measured separately according to the method of Passoneau (13). Briefly, 1 ml of 0.5 M NaOH was added to frozen tissue (~50 mg) and heated in boiling water for 20–30 min to remove tissue free glucose. The suspension was made acidic with a known volume of 12% HCl (pH < 3.0). An aliquot of acid extract (~50 μl) was removed and added to 950 μl of 200 mM acetate buffer (pH 4.7) containing amylase–l,4–l,6-glucosidase to digest tissue glycogen. Following 2 h of incubation, the acid extract was fluorometrically assayed for glucose (13).

Quantitation of NMR Spectra—31P NMR spectral intensities for the phosphorus-containing compounds were determined by computer integration using the VNMRX software. Saturation correction factors were determined by taking the ratio of the area under a given peak with a 20-s relaxation delay to the area of the spectra with a 1-s relaxation delay. The mean correction factors for the 10 mM phenylphosphonic acid external standard, P2, PCr, and β-ATP were 2.20 ± 0.05, 1.80 ± 0.06, 1.56 ± 0.0, and 1.22 ± 0.02 (± S.E., n = 18), respectively. Intracellular concentrations of the β-ATP, PCr, and P1 were calculated by equating the spectral intensities of the saturation-corrected phosphorus metabolites to the spectral intensity of the saturation-corrected external standard (10 mm).

\[
\frac{[P]}{[P]} = \frac{1.80 \times \text{area}_{\text{det}}} {2.20 \times \text{area}_{\text{std}}} \times 10 \text{ mm} \tag{1}
\]

\[
\frac{[\text{PCr}]}{[P]} = \frac{1.56 \times \text{area}_{\text{PCr}}}{2.20 \times \text{area}_{\text{std}}} \times 10 \text{ mm} \tag{2}
\]

\[
\frac{[\text{ATP}]}{[P]} = \frac{1.22 \times \text{area}_{\text{ATP}}}{2.20 \times \text{area}_{\text{std}}} \times 10 \text{ mm} \tag{3}
\]

Intracellular pH (pHi) was calculated from the chemical shift (δ ppm) of P1 relative to PCr in the 31P NMR spectra using the NMR version of the Henderson–Hasselbalch equation (1).

\[
\text{pHi} = 6.75 + \log \left( \frac{\delta - 3.25}{6.89 - \delta} \right) \tag{4}
\]

Intracellular free Mg2+ concentration ([Mg2+]i) was calculated from the observed chemical shift difference (δhi) in ppm between β-ATP and α-P resonances of ATP in the 31P NMR spectra using the modified form of the London equation (14), where \(a = [\text{H}][\text{K}\beta] / [\text{K}\alpha][\text{K}P] \).

\[
[Mg^{2+}] = K_{\text{Mg}} \left[ \frac{\delta_{\text{hi}} (1 + a) - (\delta_{\text{hi}} + \delta_{\text{hi}}) \delta_{\text{hi}} + \beta \delta_{\text{hi}} - \delta (1 + \beta)} {1 \alpha \delta_{\text{hi}} - \delta (1 + \beta)} \right] \tag{5}
\]

The parameters \(\delta_{\text{hi}}, \delta_{\text{hi}}, \delta_{\text{hi}}, \delta_{\text{hi}}\), and \(\delta_{\text{hi}}\) were assigned published values of 10.0, 6.110, 8.165, and 8.522 ppm, respectively; \(K_{\text{Mg}} = 9.0 \times 10^{-5} \text{ M} \); \(K_{\text{K}} = 3.4 \times 10^{-7} \text{ M} \); and \(K_{\text{K}} = 7.2 \times 10^{-4} \text{ M} \).

The free cytosolic [ADP] was calculated from the creatine kinase equilibrium (EC 2.7.3.2) using the measured components in the 31P NMR spectra.

\[
[\text{ADP}] = \frac{[\text{ATP}][\text{Cr}]} {[P]} \tag{6}
\]

The Cr concentration was calculated by subtracting the NMR PCr value from the total Cr measured enzymatically where the total Cr concentration \(C_{\text{Pcr}} \) in vitro was determined using a chromium titration procedure. The Cr concentration was adjusted to muscle free [Mg2+]i and pH, during graded levels of steady-state work according to the method of Golding et al. (15). In addition, \(K_{\text{Cr}} \) was adjusted to the temperature of the muscle (30 °C) using the method of Teague et al. (16).

Statistical Analysis—All values shown are means ± S.E. Unless otherwise indicated, Student’s t test was applied for statistical compar...
following 10 min of stimulation and glycogen, lactate, and pyruvate were measured enzymatically (Table I). Up to 0.5 Hz, there was no significant difference in muscle glycogen, lactate, and pyruvate compared with controls. At 0.8 Hz, a significant rise in lactate and pyruvate ($p < 0.05$) was observed with a concomitant fall in glycogen. It was not until 1 and 2 Hz that glycogen fell as low as 30% of control, lactate increased up to 5-fold, and lactate/pyruvate ratio increased 3-fold (Table I). NMR determined cytosolic pH, also reported in Table I, was not significantly different from controls up to 0.5 Hz but significantly decreased from 7.203 to 7.050 at 0.8 Hz. On the basis of these changes in TTI and accompanying metabolic data, we estimated that the maximal mitochondrial capacity in rat gastrocnemius in situ under our conditions was around 0.8 Hz.

Changes in the PCr, ATP, Cr, Free [Mg$^{2+}$], and Calculation of Free [ADP] from the Creatine Kinase Equilibrium—NMR determined PCr, ATP, P$_i$, and free Mg$^{2+}$ in rat gastrocnemius muscle in vivo during the 8–12 min of stimulation are reported in Table II. During submaximal steady-state work transitions, PCr fell in significantly different increments ($p < 0.05$) at each stimulation frequency up to 0.8 Hz (Table II). The work-induced fall in PCr was accompanied by a stoichiometric rise in intracellular P$_i$ concentration (Table II). It is noteworthy that ATP began to decrease at 2 Hz when PCr was about 30% of the control value (Table II). ATP was not considered part of our aerobic steady-state criteria because it remained constant up to and including 1.0 Hz, presumably through PCR hydrolysis and the activation of anaerobic glycolysis (Tables I and II). Free [Mg$^{2+}$] was not significantly different up to 1 Hz but increased by 50% at 2 Hz.

Free cytosolic [ADP] was 17 μM in the gastrocnemius prior to stimulation and increased with increasing stimulation frequency and tension-time integral (Fig. 2a). The equation to the line up to 0.8 Hz was $TTI = 46.984 ([ADP]) + 2.2562$ with $R^2 = 0.9945$. When free [ADP] was plotted against TTI using the Hill relationship, given a $V_{max}$ of 39 Ns at 0.8 Hz, a Hill coefficient ($n_H$) of 2.4 was found (Fig. 2b). The equation to the line was $log(TTI/V_{max} - TTI) = 2.38 (log ADP) - 4.00$ with $R^2 = 0.99$. The apparent $K_{0.5}$ of ADP for submaximal steady-state stimulation was 48 μM. A similar analysis was performed with increases in cytosolic [P$_i$]. A Hill coefficient of 2.1 was obtained. The equation to the line was $log(TTI/V_{max} - TTI) = 2.14(log[P_i]) - 2.08$ with $R^2 = 0.96$ (graph not shown). The apparent $K_{0.5}$ value of free [P$_i$] for submaximal steady-state work was 9.4 mM. The data for free cytosolic [ADP] (and free [P$_i$]) and TTI did not fit any form of the Michaelis-Menten function, i.e., the Lineweaver-Burke plot showed no tendency to intersect the y axis (Fig. 2c). Similarly, the Eadie-Hofstee and Hanes Plot followed no sensible form, leading to the calculation of a kinetic $V_{max}$ or apparent $K_m$ (plots not shown).

### RESULTS

**Establishment of Steady-state and Apparent Aerobic $V_{max}$ Of Muscle**—Steady-state was defined in terms of relatively constant TTI and concentrations of PCr and P$_i$ measured at the different stimulation frequencies in consecutive spectra acquired in 4-min blocks (Fig. 1). Mean values for TTI were similarly calculated in three blocks of 4 min to coincide with the NMR acquisition period. Fig. 1a shows no significant differences in TTI at the stimulation frequencies between 0.1 and 0.8 Hz over the 12-min time period. Significant changes were observed at 1 and 2 Hz. At all stimulation frequencies, PCr fell significantly during the first and second periods of acquisition (0–8 min) (Fig. 1b). With the exception of 1 and 2 Hz, metabolic steady state was reached during the period between 8 and 12 min. A similar profile was found with the increase in muscle P$_i$ concentration at the different stimulation frequencies (Fig. 1c).

In parallel bench experiments, muscles were freeze-clamped after 10 min of stimulation and glycogen, lactate, and pyruvate were measured enzymatically (Table I). Up to 0.5 Hz, there was no significant difference in muscle glycogen, lactate, and pyruvate compared with controls. At 0.8 Hz, a significant rise in lactate and pyruvate ($p < 0.05$) was observed with a concomitant fall in glycogen. It was not until 1 and 2 Hz that glycogen fell as low as 30% of control, lactate increased up to 5-fold, and lactate/pyruvate ratio increased 3-fold (Table I). NMR determined cytosolic pH, also reported in Table I, was not significantly different from controls up to 0.5 Hz but significantly decreased from 7.203 to 7.050 at 0.8 Hz. On the basis of these changes in TTI and accompanying metabolic data, we estimated that the maximal mitochondrial capacity in rat gastrocnemius in situ under our conditions was around 0.8 Hz.

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### DISCUSSION

There has been much controversy in recent years about whether the relationship between oxygen consumption and free [ADP] follows a first order Michaelis-Menten function or higher order sigmoidal function. Our study shows that ADP and TTI follows a first order Michaelis-Menten function or higher order sigmoidal function. Our study shows that ADP and TTI
change occurred in twitch tension, PCr, and Pi between the second and third spectra up to 0.8 Hz. Moreover, there was no significant change in muscle lactate, glycogen, and pH indicating a physiological and metabolic steady state (Tables I and II). Our study therefore differs from most in this area by experimentally determining the apparent maximum aerobic mitochondrial capacity \( V_{\text{max}} \) in situ not from fitting the data to a specific mathematical relation (8, 17–19). The third assumption, that tension-time index provides a reliable estimate of oxygen consumption, has been shown by many studies in the past for skeletal muscle (9, 10, 20–24) and heart (25, 26). For the rat hindlimb in vivo, Brindle et al. also have shown a linear function between tension-time integral and ATP turnover (23). Kushnerick and Paul also showed a linear relation between recovery oxygen consumption and TTI in frog sartorius (27).

Our Hill coefficient \( n_{\text{Hill}} \) for [ADP] and TTI of 2.4 for rat gastrocnemius in situ is consistent with the published work of 2.2 for human forearm muscle (8, 19). Prior to the work of Jeneson et al. in 1996 (8), the relationship between steady-state work and free [ADP] had been assumed in vivo to follow a Michaelis-Menten function in rat leg muscle (28), cat perfused biceps (29), and human arm muscle (30). Some of the problems associated with these older kinetic analysis have been discussed by Kemp (31) and more recently by Jeneson et al. (8, 32) with a reply from Portman et al. (33). The present study supports the idea that the relationship between oxygen consumption and free [ADP] follows at least a second order sigmoidal function.

In addition, the apparent \( K_{\text{m}} \) of [ADP] of 48 \( \mu \text{M} \) for tension time index (and oxygen consumption) we report in the present study is also consistent with some literature values. Again, this is a very controversial area. In 1989, Brindle and co-workers reported an apparent \( K_{\text{m}} \) of at least 30 \( \mu \text{M} \) for rat hindlimb in situ under steady-state submaximal exercise (23). These data have subsequently been recalculated to give an apparent \( K_{\text{m}} \) of about 50 \( \mu \text{M} \) (18, 34). Similarly, Thompson and colleagues in 1995 studied phosphocreatine recovery in rat leg muscle in vivo and reported an apparent \( K_{\text{m}} \) of ADP greater than 30 \( \mu \text{M} \). In ex vivo perfused cat biceps, Kushnerick and colleagues (29) calculated an apparent \( K_{\text{m}} \) for free [ADP] of 23 \( \mu \text{M} \), a value similar to 26 \( \mu \text{M} \) for rabbit gastrocnemius/soleus muscle groups in vivo (24). Better agreement is found with the work of Jeneson and co-workers (8), who calculated an apparent \( K_{\text{m}} \) of ADP of 44 \( \mu \text{M} \) in human forearm flexor muscle (8).

One possible concern that could be raised with our kinetic analysis is that the tension-time integral does not saturate with free [ADP] in vivo (Fig. 2a). This is to be expected in an intact muscle because of the recruitment of competing metabolic pathways and fuels to maintain ATP constant as the muscle approaches its maximal aerobic capacity. Indeed, in our study, ATP did not fall significantly until 2 Hz, which is far in excess of our estimate of aerobic capacity (25). Our system does not saturate in free [ADP] levels (36–38), [ATP]/[ADP] \( \text{[Pi]} \) ratio (39, 40), the \([\text{ATP}] / [\text{ADP}] \) linked \([\text{NADH}] / [\text{NAD}] \) ratios.

### Table I

Total tissue concentrations of lactate, glycogen, and pyruvate determined enzymatically in freeze-clamped rat gastrocnemius muscle in vivo following 10-min stimulation.

| Hz | n  | Lactate | Glycogen | Pyruvate | Lactate/pyruvate | pH |
|----|----|---------|----------|----------|------------------|----|
| 0.1| 7  | 1.76 ± 0.16 | 30.7 ± 1.6 | 0.055 ± 0.007 | 32.8 ± 4.7 | 7.20 ± 0.01 |
| 0.2| 5  | 1.81 ± 0.21 | 30.7 ± 1.4 | 0.040 ± 0.004 | 46.0 ± 8.0 | 7.14 ± 0.01 |
| 0.3| 5  | 1.66 ± 0.31 | 30.7 ± 1.6 | 0.037 ± 0.006 | 49.4 ± 11.4 | 7.15 ± 0.01 |
| 0.4| 5  | 1.28 ± 0.23 | 30.9 ± 1.6 | 0.032 ± 0.008 | 43.4 ± 5.3 | 7.14 ± 0.01 |
| 0.5| 4  | 1.61 ± 0.13 | 28.5 ± 3.9 | 0.044 ± 0.004 | 37.9 ± 4.7 | 7.12 ± 0.01 |
| 0.6| 5  | 2.57 ± 0.18 | 25.6 ± 3.9 | 0.077 ± 0.005 | 33.5 ± 2.1 | 7.05 ± 0.04 |
| 1  | 7  | 4.32 ± 0.84 | 21.6 ± 2.5 | 0.066 ± 0.011 | 59.9 ± 3.1 | 7.01 ± 0.04 |
| 2  | 5  | 9.35 ± 1.42 | 9.3 ± 0.18 | 0.095 ± 0.007 | 100.5 ± 18.1 | 6.84 ± 0.06 |

### Table II

Intracellular concentrations of PCr, ATP, Mg, ADP, and P, during 8–12 min of stimulation in rat gastrocnemius muscle in vivo using \(^{31}\text{P NMR spectroscopy}\).

| Hz | n  | [TCr] | [PCr] | [Cr] | [ATP] | [Mg] | \( K_{\text{cr}} \) | [ADP] | [P] |
|----|----|-------|-------|------|-------|------|-------------|-------|-----|
| 0.1| 7  | 29.96 ± 0.53 | 38.78 ± 0.52 | 7.22 ± 0.52 | 8.99 ± 0.11 | 0.58 ± 0.01 | 104.0 ± 3.3 | 17.0 ± 1.8 | 2.80 ± 0.19 |
| 0.2| 5  | 29.72 ± 0.74 | 35.78 ± 0.55 | 10.22 ± 0.55 | 9.03 ± 0.13 | 0.59 ± 0.08 | 112.5 ± 3.7 | 23.4 ± 1.4 | 3.94 ± 0.31 |
| 0.3| 5  | 29.34 ± 0.65 | 32.25 ± 0.59 | 13.75 ± 0.53 | 9.02 ± 0.11 | 0.61 ± 0.02 | 120.9 ± 3.4 | 32.6 ± 2.0 | 6.76 ± 0.51 |
| 0.4| 5  | 30.69 ± 0.73 | 28.78 ± 0.59 | 17.22 ± 0.59 | 9.02 ± 0.10 | 0.64 ± 0.04 | 121.5 ± 3.4 | 45.9 ± 2.9 | 8.71 ± 0.68 |
| 0.5| 5  | 30.37 ± 0.38 | 23.89 ± 0.84 | 22.11 ± 0.84 | 8.67 ± 0.08 | 0.60 ± 0.02 | 127.3 ± 3.6 | 65.2 ± 3.7 | 11.91 ± 0.75 |
| 0.6| 5  | 31.08 ± 0.64 | 18.98 ± 1.00 | 27.02 ± 1.00 | 8.58 ± 0.18 | 0.58 ± 0.04 | 144.6 ± 3.6 | 86.3 ± 5.7 | 15.57 ± 1.56 |
| 1  | 7  | 27.41 ± 0.44 | 17.08 ± 1.13 | 24.92 ± 1.13 | 8.66 ± 0.11 | 0.65 ± 0.04 | 167.6 ± 16.8 | 80.5 ± 6.0 | 16.78 ± 0.57 |
| 2  | 5  | 25.93 ± 0.92 | 11.16 ± 0.94 | 28.84 ± 0.94 | 6.26 ± 0.28 | 0.86 ± 0.09 | 287.1 ± 7.5 | 66.7 ± 8.9 | 21.07 ± 1.63 |
that free cytosolic [P] fulfils the same criteria. During increasing steady-state work, P increased 8-fold from 3 to 16 mM up to 0.8 Hz (Figs. 1 and 2 and Table II). The apparent K of 9 mM was calculated to be 9 mM and followed at least a second order relationship with tension time index. Our apparent K for [P] of 9 mM for rat gastrocnemius is lower than the 19 mM reported for rabbit gastrocnemius (24). In our study neither change in free ADP or P concentration with muscle work fit a Michaelis-Menten relationship. As Jeneson et al. (32) pointed out, the possibility also exists that the apparent overall kinetic order of the ADP-TTI relationship may also reflect [P] stimulation of respiration because in our model P was found to lie within its apparent K of 9.0 mM.

Another very interesting study relevant to our work is that of Arnold and Kadenbach (38), who in 1999 showed a sigmoidal relationship (average Hill coefficient of 2.9) between [ADP] and the activity of cytochrome c oxidase. From this relation, they suggested the possibility of regulation of respiration by intramitochondrial [ADP] (38). These authors, however, did not dismiss a role for [ADP] in the regulation of NADH dehydrogenase and cytochrome c reductase in the overall pacemaker of cell respiration (38). One difference between our study and theirs was Arnold and Kadenbach determined a matrix half-maximal ADP stimulation of cytochrome c oxidase of 170 μM (38). This value is over 3 times the “cytosolic” apparent K of [ADP] we report for tension-time index (and presumably oxygen consumption). The highest cytosolic [ADP] we measured up to 0.8 Hz stimulation frequency was around 80 μM (Table II). Arnold and Kadenbach do, however, note that mitochondrial membrane potential and the electrogenility of the nature of the ATP/ADP carrier would result in a lower matrix ATP/ADP ratio due to higher [ADP], which they argue would be consistent with their kinetic work. We therefore conclude on the basis of our data that ADP and P, could potentially control incremental increases in steady-state work in skeletal muscle in vivo.

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