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Quasi-linear relationship between Gibbs free energy of ATP hydrolysis and power output in human forearm muscle

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Laboratory for Metabolic Diseases, University Children's Hospital, 3512 LK Utrecht; NMR Laboratory, Department of Cardiology, University Hospital, 3584 CX Utrecht; Inter-University Cardiology Institute of the Netherlands; Division of Molecular Biology, H5, the Netherlands Cancer Institute, and E. C. Slater Institute, Biocentre, University of Amsterdam, 1018 TV Amsterdam, the Netherlands; and Department of NMR and Medical Spectroscopy, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111

Jenerson, J. A. L., H. V. Westerhoff, T. R. Brown, C. J. A. van Echteld, and R. Berger. Quasi-linear relationship between Gibbs free energy of ATP hydrolysis and power output in human forearm muscle. *Am. J. Physiol.* 268 (*Cell Physiol.* 37): C1474–C1484, 1995.—The postulated strictly linear descriptions of the rate dependence of oxidative phosphorylation in skeletal muscle on the free energy of ATP hydrolysis (ΔG_P) over the range of physiological steady states fail to harmonize with reported findings of identical basal respiration rates in mammalian muscles at different ΔG_P values. The relevance of an extension of the strictly linear description to a description deriving from enzyme kinetics that predicts a sigmoidal dependence was investigated in human finger flexor muscle using ^{31}P -nuclear magnetic resonance spectroscopy. At constant pH 7.0, the experimental variation of adenine nucleotide concentrations with power output, which reflects the rate of oxidative phosphorylation, was compared with predictions by various formulations of adenine nucleotide control of respiration. The quasi-linear sigmoidal description was found to be statistically equivalent but physiologically superior to the strictly linear description. The predicted maximal oxidatively sustained steady-state power output and rate-dependent sensitivity of respiration to changes in ΔG_P were in agreement both with theoretical considerations and with experimental observations in the present study and other studies of intact mammalian skeletal muscle.

cellular energetics; respiratory control; skeletal muscle; thermodynamics

A LARGE BODY OF WORK has been published on the control of mitochondrial respiration and ATP synthesis. Several potential regulators (i.e., metabolites and thermodynamic driving forces) and the mechanism of their action have been described (for a review, see Ref. 4). Different groups have championed different primary regulators and mechanisms of action [e.g., phosphate (25) vs. redox (24) potential, kinetic (5, 6, 20, 34) vs. thermodynamic (11, 25, 40) control, and near-equilibrium (11) vs. nonequilibrium thermodynamics (40)]. The concept of distributed control among different steps in a metabolic pathway (12, 40) offers an alternative way to address this issue. Comprehensive quantitative descriptions of the contribution of each potential regulator to the overall control of oxidative phosphorylation are currently offered by the theoretical frameworks of the “mosaic nonequilibrium thermodynamics” (MNET) description of oxidative phosphorylation (40) and metabolic control theory (for a review, see Ref. 12).

With respect to mitochondrial function and its control in intact skeletal muscle under normoxic conditions, there has been general agreement on dominant control over redox state by some function of cellular adenine nucleotide levels (4). The observation of net oxidation of mitochondrial NADH during twitch stimulation under these conditions was in agreement with this assumption (22, 30). Any contribution to control by proton leak (14, 15, 40) has not been addressed in this description. The nature of the driving function of adenine nucleotides has been the subject of some debate (4). In his analysis of the phosphate energy system in creatine-containing cells, Connnett (7) showed that no unique driving function of adenine nucleotides can be identified from experiments in which only the total energy charge is varied independently. Results of a study in which pH was additionally varied were in agreement with phosphate potential [$\Delta G_P = \Delta G_P^0 + RT \ln([ATP]/[ADP][P_i])$], where ΔG_P^0 is the standard Gibbs energy change of the reaction, R is the universal gas constant, and T is absolute temperature] but not [ADP] as an independent regulator of respiration (8).

Linear mitochondrial driving functions involving thermodynamic control of respiration by ΔG_P have been put forward and tested on intact skeletal muscle preparations (7, 8, 29). In these models, a linear dependence of the rate of oxidative phosphorylation on ΔG_P was assumed over the full range of physiological metabolic states. This assumption is, however, inconsistent with reported findings of identical basal oxygen consumption rates of animal muscles with widely different ΔG_P values (9, 26, 27). These findings suggested that a strictly linear description of the rate dependence of respiration on ΔG_P may be inadequate for mammalian skeletal muscle.

An alternative description of oxidative phosphorylation in terms of thermodynamic driving forces predicts a sigmoidal relationship between the rate of respiration or phosphorylation and ΔG_P values, with an extensive quasi-linear region around the inflection point and constant regions at high and low ΔG_P (28, 33, 40). This description is an extension of the strictly linear description and a hybrid of enzyme kinetics and thermodynamics. Its basis lies in the inherent sigmoidal character of the function describing the variation of rate with driving force ΔG for any enzyme-catalyzed reaction under the boundary condition that the sum of substrates and products of the reaction is constant, with the rate

approaching maximal and minimal values at extreme values of ΔG (28, 33, 40). In agreement with this description, linear, exponential, and sigmoidal relationships between rate and ΔG , depending on the range of respiration rates studied, have been found in studies of isolated mitochondria for various chemiosmotic processes (18, 20, 25, 28, 39, 40).

In a previous study we showed that the expected relationship between ΔG_p and a measure of the rate of oxidative phosphorylation in skeletal muscle under steady-state conditions at constant pH 7.0, muscle power output, is sigmoidal (41). In this paper, we investigate the experimental relationship between ΔG_p and muscle power output in intact human forearm muscle using ^{31}P -nuclear magnetic resonance (NMR) spectroscopy (^{31}P -MRS). We found that the experimental data can be explained by a sigmoidal relationship between ΔG_p and the steady-state rate of oxidative phosphorylation. We discuss the physiological relevance of the difference between the quasi-linear (sigmoidal) and the strictly linear descriptions.

METHODS

Design of the Study

P_i and phosphocreatine (PCr) levels and pH in human finger flexor muscle (flexor digitorum profundus muscle; FDP) of the right forearm were measured by ^{31}P -MRS at four steady states (resting state and during exercise at 3 normalized power output levels). The variation of adenine nucleotide concentrations with power output was analyzed for the pooled data. To this end, mathematical equations predicting the theoretical variation of cellular adenine nucleotide concentrations with power output were formed based on thermodynamic and kinetic mitochondrial driving functions and were fitted to the experimental data. In two subjects, an additional series of measurements at eight and nine power output levels was performed for the analysis of the covariation in individual muscles, thereby eliminating any interference by biological intersubject variability that might have affected the analysis of the pooled data.

Subjects

Twenty untrained healthy subjects (15 male, 5 female), 12–42 yr old (mean age 22 yr), participated in the study. All subjects were right handed.

^{31}P -MRS

^{31}P -NMR spectra of the FDP were obtained at 1.5 T on a Philips S15 HP whole body NMR spectrometer, as described in detail elsewhere (21). Briefly, subjects were positioned prone and head first on the patient bed with their right forearm extended forward, supported by cushions. Guided by palpation of the ulnar bone directly adjacent to the muscle, the forearm was placed into a support such that the FDP overlaid a two-turn 25-mm-diameter surface coil tuned to the ^{31}P frequency (25.86 MHz) and was attached with Velcro strips. Correct positioning of the FDP over the ^{31}P surface coil was objectified by ^1H magnetic resonance imaging. ^{31}P -NMR spectra of the superficial region of the FDP were obtained with a frequency-modulated adiabatic 90° excitation pulse. Sixty free induction decays (FIDs; 1,024 data points with 333- μs dwell time) were acquired in the resting state and at each steady-state work load during exercise with a repetition time of 3 s.

Radio-frequency pulsing during exercise was gated to the audio signal that synchronized bulb-squeezing using a home-built audio-triggering device. To minimize motion artifacts and improve standardization of the measurements, the radio-frequency pulse was set to occur 1,250 ms after contraction.

Data Processing

The FIDs were processed using the LAB ONE (New Methods Research) NMR1 spectroscopy processing software, as described in detail elsewhere (21). Estimates of the relative peak areas of the various metabolites were obtained by curve fitting of the spectrum to Lorentzian line shapes (P_i and PCr, both singlets; γ -, α -, and β -ATP, 2 doublets and a triplet, respectively). Intracellular pH was determined from the chemical shift of P_i relative to PCr, which was set at 0.00 ppm (35). Any regional variation of pH within the sampled muscle mass was determined by examination of the P_i peak and comparison of its width with that of the PCr peak, as determined by the fitting routine.

Calculation of Metabolite Concentrations

The concentrations of P_i and PCr in the resting state were calculated from measured $\text{P}_i/\beta\text{-ATP}$ and $\text{PCr}/\beta\text{-ATP}$, assuming a constant ATP concentration of 8.2 mM (16). To correct for partial saturation associated with the repetition time of 3 s, $\text{P}_i/\beta\text{-ATP}$ and $\text{PCr}/\beta\text{-ATP}$ were determined in measurements with a repetition time of 20 s ($n = 3$) and compared with those at 3 s, yielding correction factors of 1.2 ± 0.1 and 1.4 ± 0.1 (SD), respectively. During exercise, P_i and PCr concentrations were calculated from the measured relative changes in P_i and PCr from resting levels. The average free ADP concentration in fibers within the sampled muscle mass was calculated from the creatine kinase equilibrium according to the equation

$$[\text{ADP}] = [\text{ATP}][\text{creatine}]/(1.66 \times 10^9)(10^{-\text{pH}})[\text{PCr}] \quad (1)$$

where $1.66 \times 10^9 \text{ M}$ is the value of the equilibrium constant (37). Two approaches were used to obtain a value for the total creatine concentration ($[\text{TCr}]$) to calculate the free creatine concentration ($[\text{Cr}]$): 1) assumption of a constant value for $[\text{TCr}]$ of 42.7 mM as reported for human leg muscle (16) and 2) calculation of individual $[\text{TCr}]$ values for each subject using the measured individual PCr level in the resting state based on the assumption that the percentage of phosphorylated creatine of the total creatine pool, the $[\text{PCr}]/[\text{TCr}]$, in the resting state was the same in all subjects. A value of 0.85 was used, calculated from the mean $[\text{PCr}]$ for this group of subjects (36.5 mM) and the reported value for the average $[\text{TCr}]$ in human leg muscle (42.7 mM) (16).

Exercise

Exercise consisted of bulb-squeezing on an audio signal (frequency, 0.33 Hz; duration, 300 ms) using only the fourth and fifth digits at progressively increasing submaximal work loads in a ramp protocol with feedback of power output (details described in Ref. 21). Power output was measured and recorded as $\int \text{P} \cdot \text{dv}$ (developed pressure times displaced volume of air) and normalized to power output during maximal voluntary contraction (MVC). In each study, the implemented work loads were carefully set to meet the subject's oxidative capacity by on-line monitoring of the metabolic response to each work load, based on our reproducible finding that significant anaerobic glycolysis and the associated steep drop in pH to < 6.90 in exercising forearm muscle only occurs after $> 60\%$ depletion of the initial PCr pool, similar to findings reported by others (35). The highest implemented work load was $\sim 60\%$ MVC based on our empirical finding of significant line broadening of P_i and

decline of pH to <6.90 at higher work loads in this group of subjects.

Fits of linear and nonlinear functions to experimental data were computed by a nonlinear least-squares routine using standard software (Fig.P version 6.0; Elsevier Biosoft, Cambridge, UK). Computed values for parameters of functions are given $\pm$ SE from regression and the degrees of freedom (DF). A value for the goodness-of-fit index (GFI; range 0–1), reflecting the probability that the fit was not due to random fluctuations in the data, is given for the fits. Statistical comparisons were made using a paired Student's *t*-test.

RESULTS

Variation of Cellular Adenine Nucleotide Concentrations With Power Output: Theoretical Steady-State Relations

Theoretical steady-state relations between muscle power output and functions of cytosolic adenine nucleotide concentrations are formulated and are compared with the experimental steady-state relations. The concept of ATP homeostasis at steady state and thermodynamic and kinetic formulations of adenine nucleotide control of respiration formed the basis for the formulation of the theoretical relations. Sigmoidal and linear relationships between power output and the Gibbs free energy of ATP hydrolysis, ΔG_P , are derived (Eqs. 9 and 10), as well as a hyperbolic relation between power output and the free ADP concentration (Eq. 11).

Cellular ATP consumption. Let J_P^b be the basal rate of ATP consumption in skeletal muscle in the resting state. In a study of the energetic cost of tension production by insect flight muscle, additional ATP consumption by myosin adenosinetriphosphatase (ATPase) during isotonic contractions varied linearly with varying mechanical power output (P , work per unit time) (32). Similarly, a linear relationship between ATP turnover rate and the force·rate product has been reported for isometric contractions (19, 32). Hence, the rate of cellular ATP consumption, J_P^C , in isotonically contracting muscle can be described as

$$J_P^C = J_P^b + \alpha \cdot P \quad (2)$$

where α is the proportionality constant relating the myosin ATP consumption rate J_P^{myosin} and mechanical power output P . To simplify the algebra, we arbitrarily set the value of α at 1 in our modeling.

Cellular ATP production. Total cellular ATP production is the sum of mitochondrial ATP production (J_P^{Ox}) and glycolytic ATP production (J_P^{Gly}). In view of the small (5%) contribution of the latter to total ATP synthesis in the presence of oxidative phosphorylation, we shall focus on the former. Because only the total of high-energy phosphate bonds was independently varied in the experiments, both thermodynamic and kinetic mitochondrial driving functions are considered in the modeling (7).

Thermodynamic control of J_P^{Ox} by ΔG_P . Because of covariation of substrate and product concentrations, or other boundary conditions, the equation relating the rate of a simple enzyme-catalyzed reaction to its substrate and product concentrations can be translated into

an equation relating the rate of the reaction to the free energy difference across it (Eq. 2.74 in Ref. 40)

$$v = v_s \cdot \frac{e^{\Delta G/RT} - 1}{e^{\Delta G/RT} + v_s/v_p} \quad (3)$$

The function $v(\Delta G)$, the so-called “flow-force relation,” is sigmoidal with v approaching maximal and minimal values for the given covariation of substrate and product concentration (v_s and v_p , respectively) at extreme values of ΔG (28, 33, 40). We previously reported that a sigmoidal $v(\Delta G)$ relationship should also be expected for oxidative phosphorylation in skeletal muscle cells (41). This function exhibits an inflection point at which the relationship between v and ΔG is exactly linear (28, 40). Over 75% of the range of v around the inflection point, the relationship is quasi-linear, i.e., it can be adequately approximated by a linear function. This forms the basis for the previously postulated strictly linear, essentially phenomenological descriptions of the rate dependence of oxidative phosphorylation in skeletal muscle on ΔG_P (7, 29). Alternatively, the mechanistic MNET description of mitochondrial oxidative phosphorylation (40) proposes a set of linear equations that, to a close approximation, should relate the rate of each chemiosmotic process (J_i) to the change in free energy of that process (ΔG_i) for each of the three processes involved (substrate oxidation coupled to proton pumping, ADP phosphorylation coupled to proton backflow, and passive proton leaking, respectively). Importantly, the MNET description uses “elemental” coefficients related to the mechanisms and kinetics of these processes rather than phenomenological coefficients. We therefore felt it important to introduce this alternative, mechanistic linear description of the rate dependence of oxidative phosphorylation on its thermodynamic driving forces.

Under steady-state conditions in which no further net proton flow occurs ($J_{H\text{total}} = 0$), a linear equation relating J_P^{Ox} to the two driving forces (the redox and phosphate potential, ΔG_O and ΔG_P , respectively) is obtained (adapted from Eq. 3.68 in Ref. 40)

$$J_P^{\text{Ox}} = - \frac{n_H^{\text{O}} \cdot n_H^{\text{P}*} \cdot \gamma_H^{\text{P}} \cdot L_O^* \cdot L_P^*}{(n_H^{\text{O}})^2 \cdot \gamma_H^{\text{O}} \cdot L_O^* + L_H^1 + (n_H^{\text{P}*})^2 \cdot \gamma_H^{\text{P}} \cdot L_P^*} \cdot (\Delta G_O^{\text{ex}} - \Delta G_O^{\#}) \quad (4)$$

$$+ \frac{[L_H^1 + (n_H^{\text{O}})^2 \cdot \gamma_H^{\text{O}} \cdot L_O^*] \cdot L_P^*}{(n_H^{\text{O}})^2 \cdot \gamma_H^{\text{O}} \cdot L_O^* + L_H^1 + (n_H^{\text{P}*})^2 \cdot \gamma_H^{\text{P}} \cdot L_P^*} \cdot (\Delta G_P^{\text{ex}} - \Delta G_P^{\#})$$

where L_i is the proportionality constant relating the flux J_i through an autonomous process i to the force ΔG_i across the process, γ_x^y is the asymmetry coefficient of dependence of v_x on ΔG_y , n_H^{O} is the $\text{H}^+:\text{O}$ stoichiometry of the redox proton pump, n_H^{P} is the $\text{H}^+:\text{ATP}$ stoichiometry of the $\text{H}^+:\text{ATPase}$ ($n_H^{\text{P}*}$ includes the protons involved in adenine nucleotide and phosphate translocation), ΔG_i^{ex} is ΔG_i measured outside the mitochondrion, and $\Delta G_i^{\#}$ is a constant marking the onset of the linear domain of the flow-force relationship (40). The asterisk in L_i^* denotes

the included contribution of the resistance for substrate movement across the mitochondrial inner membrane associated with ΔG^{ex} (40).

Kinetic control of J_P^{Ox} by substrate concentrations. Under conditions of saturating concentrations of mitochondrial NADH and O_2 , the overall rate of oxidative phosphorylation, as reflected in the steady-state rate of O_2 consumption (J_{O}), has been described by a rate equation for the simplest bisubstrate Michaelis-Menten kinetics (34)

$$J_{\text{O}} = J_{\text{O}}^{\text{max}} \cdot 1 / (K_{\text{i}}^{\text{a}} K^{\text{b}} / [\text{ADP}][\text{P}_{\text{i}}] + K^{\text{a}} / [\text{ADP}] + K^{\text{b}} / [\text{P}_{\text{i}}] + 1) \quad (5)$$

where $J_{\text{O}}^{\text{max}}$ is the maximal rate of respiration; K^{a} and K^{b} are the limiting Michaelis constants for ADP and P_{i} , respectively; and K_{i}^{a} is the inhibition constant for ADP (34). The rates of the reverse reaction (mitochondrial ATP hydrolysis) and of any net forward reaction in the absence of ADP or phosphate (due to uncoupling) are neglected in this description. Also, product inhibition by ATP is assumed to be absent. In fully coupled mitochondria

$$J_{\text{P}} = J_{\text{O}} \cdot 2n_{\text{P}}^{\text{O}} \quad (6)$$

where J_{P} is the rate of ATP production and n_{P}^{O} is equivalent to the number of moles of ADP phosphorylated per one-half mole of O_2 consumed. This so-called P/O is equivalent to the ratio of n_{H}^{O} to n_{H}^{P} in Eq. 4.¹ Substitution of Eq. 6 into Eq. 5 yields the following equation relating the rate of mitochondrial ATP production to the concentrations of the substrates ADP and P_{i}

$$J_{\text{P}} = J_{\text{O}}^{\text{max}} \cdot 2n_{\text{P}}^{\text{O}} \cdot 1 / (K_{\text{i}}^{\text{a}} K^{\text{b}} / [\text{ADP}][\text{P}_{\text{i}}] + K^{\text{a}} / [\text{ADP}] + K^{\text{b}} / [\text{P}_{\text{i}}] + 1) \quad (7)$$

ATP homeostasis during contraction: steady-state relations. During cellular ATP consumption under normoxic steady-state conditions and when any contribution of glycolytic ATP production to cellular ATP production is negligible (the condition of constant pH 7.0 in Connett's analysis; Ref. 7), the rate of ATP consumption equals the rate of ATP production by oxidative phosphorylation, hence

$$J_{\text{P}}^{\text{b}} + P = J_{\text{P}}^{\text{Ox}} \quad (8)$$

Substitution of the function describing the sigmoidal dependence of J_{P}^{Ox} on ΔG_{P} (adapted from Eq. 3) into Eq. 8 yields the following sigmoidal relation between steady-state power output P and the cytosolic concentrations of ATP, ADP, and P_{i} of the form

$$P = P_{\text{max}} \cdot \frac{e^{(n/C) \ln([\text{ATP}]/[\text{ADP}][\text{P}_{\text{i}}])} - 1}{e^{(n/C) \ln([\text{ATP}]/[\text{ADP}][\text{P}_{\text{i}}])} + P_{\text{max}}/P_{\text{min}}} \quad (9)$$

where P_{max} is the maximal steady-state power output level (in %MVC) for the given boundary conditions of substrate and product concentrations and redox potential. The basal rate, J_{P}^{b} in Eq. 8, is reflected in the value of P_{min} (in %MVC) relative to the resting state. The function parameters n and C are constants describing the quasi-linear domain of the curve traversing from P_{max} to P_{min} (n is a measure of the slope and C is $\sim \Delta G_{\text{P}}^{\#}$ in Eq. 4, the abscissa). The quasi-linear domain of this function can be approximated by the linear relation between P and $\ln([\text{ATP}]/[\text{ADP}][\text{P}_{\text{i}}])$ that is obtained by substitution of the MNET equation given by Eq. 4 under conditions of constant redox potential into Eq. 8

$$P = m \cdot \ln([\text{ATP}]/[\text{ADP}][\text{P}_{\text{i}}])^{\text{ex}} + b \quad (10)$$

where

$$m = \frac{RT [L_{\text{H}}^{\text{I}} + (n_{\text{H}}^{\text{O}})^2 \cdot \gamma_{\text{H}}^{\text{O}} \cdot L_{\text{O}}^{\text{I}}] \cdot L_{\text{P}}^{\text{I}}}{[(n_{\text{H}}^{\text{O}})^2 \cdot \gamma_{\text{H}}^{\text{O}} \cdot L_{\text{O}}^{\text{I}} + L_{\text{H}}^{\text{I}} + (n_{\text{H}}^{\text{P}})^2 \cdot \gamma_{\text{H}}^{\text{P}} \cdot L_{\text{P}}^{\text{I}}]}$$

and b is a constant containing $\Delta G_{\text{P}}^{\#}$, $\Delta G_{\text{O}}^{\text{O}}$, $\Delta G_{\text{O}}^{\text{ex}}$, $\Delta G_{\text{O}}^{\text{I}}$, J_{P}^{b} , and physical constants related to mitochondrial functionality (see Eq. 4).

For kinetic control of J_{P}^{Ox} by the cytosolic concentrations of the substrates ADP and P_{i} under conditions of saturating concentrations of mitochondrial NADH and O_2 , we obtain a hyperbolic relationship between steady-state power output P and the cytosolic concentration of ADP and P_{i} by substituting Eq. 7 for J_{P}^{Ox} into Eq. 8. In human skeletal muscle, however, the $[\text{ADP}]$ sensitivity of respiration is dominant over $[\text{P}_{\text{i}}]$ in view of the resting state concentration of each metabolite relative to its Michaelis constant (e.g., 1, 3, 5) and the covariant increase of $[\text{ADP}]$ and $[\text{P}_{\text{i}}]$ during contractions (Ref. 7; see the APPENDIX). As a result, the hyperbolic function describing the relation between muscle power output and the concentration of adenine nucleotides can be simplified to the following equation

$$P = C_1 \cdot 1 / (K_{\text{m}} / [\text{ADP}] + 1) - C_2 \quad (11)$$

where $C_1 = J_{\text{O}}^{\text{max}} \cdot 2n_{\text{P}}^{\text{O}}$, $C_2 = J_{\text{O}}^{\text{b}} \cdot 2n_{\text{P}}^{\text{O}}$, and K_{m} is the apparent Michaelis constant for $[\text{ADP}]$ stimulation of respiration.

Variation of Cellular Adenine Nucleotide Concentrations With Power Output: Experimental Data

Resting state. The means $\pm$ SD and ranges of concentrations of relevant metabolites and $\ln([\text{ATP}]/[\text{ADP}][\text{P}_{\text{i}}])$ in the FDP of 20 subjects determined in the resting state are given in Table 1. Values for $[\text{ADP}]$ were calculated according to two approaches as described in METHODS. For comparison, mean values for $[\text{PCr}]$ and $[\text{PCr}]/[\text{TCr}]$ ($\pm$ SD) calculated assuming values of 8.2 and 42.7 mM for $[\text{ATP}]$ and $[\text{TCr}]$, respectively, in two previous ^{31}P -MRS studies of human forearm flexor muscle were 38.3 ± 2.0 and 0.9 ± 0.1 , respectively ($n = 20$ subjects; Ref. 1), and 32.8 ± 4.7 and 0.8 ± 0.1 , respectively ($n = 8$ subjects; Ref. 2).

Variation of adenine nucleotide concentrations with power output: pooled data. Figure 1 shows a typical

¹ It should be noted that in this kinetic description this "P/O" is taken to be constant. In the nonequilibrium description, however, the actual P/O ($J_{\text{P}}/J_{\text{O}}$) is allowed to vary (40).

Table 1. Metabolite concentrations in forearm muscle in the resting state, estimated from ³¹P-NMR spectra of the flexor digitorum profundus muscle of 20 subjects

	Means ± SD*	Range*	Means ± SD†	Range†
pH _i	7.06 ± 0.02	7.02–7.09		
P _i , mM	3.3 ± 0.3	2.5–3.9		
PCr, mM	36.5 ± 2.7	32.8–41.4		
TCr, mM	42.7		42.9 ± 3.3	38.6–48.7
Cr, mM	5.7 ± 2.7	1.3–9.9	6.5 ± 0.5	5.8–7.3
ADP, μM	9 ± 5	2–17	10 ± 1	9–11
ln([ATP]/[ADP][P _i]), M ⁻¹	12.7 ± 0.6	12.0–14.0	12.5 ± 0.1	12.3–12.7

Values are means ± SD and range for 20 subjects. P_i and phosphocreatine (PCr) concentrations were calculated from relative peak areas over β-ATP assuming [ATP] = 8.2 mM (16). [ADP] was calculated assuming equilibrium of creatine kinase reaction (37). *Calculated assuming total creatine pool (TCr) of 42.7 mM (16). †Calculated assuming percentage phosphorylated creatine (PCr) of total creatine pool is 85% in resting state.

series of ³¹P-NMR spectra obtained from the FDP in the resting state and at three normalized, progressively increasing power output levels. In all subjects, intracellular pH remained within the interval of 7.12–6.90 and homogeneous throughout the sampled muscle mass over the studied range of power output levels (as found by analysis of the line widths of the P_i and PCr peaks in each ³¹P-NMR spectrum, and illustrated in Fig. 1). The condition of constant pH 7.0 for applicability of the analysis (7; see above) was thus met within 0.1 pH units. The average [ADP] in fibers was calculated according to approach 2 described in METHODS. Figure 2 shows the variation of the concentration-dependent term of the Gibbs free energy of ATP hydrolysis, ln([ATP]/[ADP][P_i]), with muscle power output for the pooled data of the 20 subjects. As shown by the dashed line in Fig. 2, the covariation was adequately described by the sigmoidal function given by Eq. 9 relating steady-state power output to cellular adenine nucleotide concentra-

Fig. 2. Variation of adenine nucleotide concentrations (M) in fibers within superficial region of FDP, reflected in value of ln([ATP]/[ADP][P_i]), with power output (%MVC) (pooled data of 20 subjects). ADP and P_i concentrations were calculated from measured PCr and P_i levels, respectively, as described in METHODS. Dashed and solid lines represent fit of Eqs. 9 and 10, respectively, to data. Regression equations are as follows: dashed line, $P = 71(e^{(9.50 - 0.76 \cdot \ln([ATP]/[ADP][P_i]) - 1)} / (e^{(9.50 - 0.76 \cdot \ln([ATP]/[ADP][P_i]) - 1)} + 3.9))$; solid line, $P = -14.9 \cdot \ln([ATP]/[ADP][P_i]) + 185$.

tions (GFI = 0.42). The estimates for P_{max}, P_{min}, n, and C were 71 ± 18% MVC, 18 ± 18% MVC, 0.76 ± 0.38, and 12.5 ± 0.1 M⁻¹, respectively (84 DF). These results show that under steady-state conditions, the variation of power output with phosphorylation potential follows the theoretical expectation reported previously (41). Furthermore, Fig. 2 suggests that the variation also follows the linear approximation proposed by MNET (40). To substantiate this, the linear function given by Eq. 10 was fitted to the ln[ATP]/[ADP][P_i] vs. power output data (Fig. 2, solid line). A highly significant linear correlation between ln([ATP]/[ADP][P_i]) and power output was found (r² = 0.88; GFI = 0.48). The estimates for the slope and the intercept were -14.9 ± 0.6% MVC · M and 185 ± 6% MVC, respectively.

Fig. 1. Typical series of ³¹P-nuclear magnetic resonance spectra of superficial mass of flexor digitorum profundus (FDP) obtained from a subject at rest and during combined flexion of fourth and fifth digits at 3 increasing power output levels in a ramp protocol. Note absence of any broadening of P_i resonance relative to phosphocreatine (PCr), indicating homogeneity of intracellular pH among fibers within sampled muscle mass at each power output level. Intracellular pH at 68% maximum voluntary contraction (MVC) power output was 6.95.

Fig. 3. Variation of ADP concentration (μM) in fibers in superficial region of FDP with power output (%MVC) (pooled data of 20 subjects). Solid line represents fit of Eq. 11 to data. Regression equation: $P = 120 / (1 + 37 / [\text{ADP}]) - 25$. Inset: predicted (Ref. 7; see APPENDIX) linear covariation of ADP and P_i concentrations (μM and mM , respectively) in fibers over range of power output levels studied. Solid line shows regression: $[P_i] = 0.19 \cdot [\text{ADP}] + 1.8$.

In line with expectations for creatine-containing cells (Ref. 7; see also the APPENDIX), a linear covariation of $[P_i]$ and $[\text{ADP}]$ was found over the studied range of steady-state power output levels ($r^2 = 0.75$; Fig. 3, inset). The estimates of the slope and intercept of the linear function describing the covariation were 0.19 ± 0.01 and $1.8 \pm 0.5 \text{ mM}$, respectively. Consequently, the rate dependence of oxidative phosphorylation on both $[\text{ADP}]$ and $[P_i]$ (Eqs. 4 and 5) effectively reduced to a single variable dependence, thereby rendering any adenine nucleotide mitochondrial driving function not unique (7). This was also illustrated by the finding that the variation of adenine nucleotide concentrations with power output could also be adequately described by the shifted single substrate Michaelis-Menten rate equation (Eq. 11) relating $[\text{ADP}]$ and power output (Fig. 3; GFI = 0.72). The estimates for C_1 , K_m^{ADP} , and C_2 (85 DF) were $120 \pm 8\%$ MVC, $37 \pm 11 \mu\text{M}$, and $25 \pm 5\%$ MVC, respectively. The estimate for K_m^{ADP} was in agreement with the range of reported values ($6\text{--}50 \mu\text{M}$) for isolated mitochondria (6, 20, 34) and perfused skeletal muscle (26).

Variation of adenine nucleotide concentrations with power output: individual muscles. Figures 4–6 show the results of studies conducted on two subjects in which the steady-state phosphate metabolite levels and pH were measured at six and seven power output levels, respectively, in a ramp protocol (corresponding data points in Figs. 4–6 are indicated by open squares). Following the measurement at the highest power output level, two additional measurements were subsequently performed at lower levels of steady-state power output to investigate any hysteresis (corresponding data points in Figs. 4–6 are indicated by open circles). In both subjects, intracellular pH remained within the interval of 7.12–7.00 and homogeneous throughout the sampled muscle mass over the range of power output levels studied; only in the spectrum obtained from *subject A* at 64% MVC

power output was some (30%) line broadening of the P_i peak relative to the line width of the PCr peak observed. The findings were similar for both subjects, as well as similar to the findings for the pooled data.

Figure 4 shows the variation of $\ln([\text{ATP}]/[\text{ADP}][P_i])$ with muscle power output for *subject A*. Hysteresis was absent. The sigmoidal function given by Eq. 9 adequately described the covariation (dashed line; GFI = 0.48). The estimates for P_{max} , P_{min} , n , and C were $66 \pm 14\%$ MVC, $18 \pm 30\%$ MVC, 1.0 ± 0.7 , and $12.4 \pm 0.4 \text{ M}^{-1}$, respectively (6 DF). The linear function given by Eq. 10 likewise adequately described the covariation (solid line) ($r^2 = 0.93$; GFI = 0.52). The estimates for the slope and the intercept were $-16.2 \pm 1.6\%$ MVC $\cdot \text{M}$ and $204 \pm 17\%$ MVC, respectively (8 DF). Similar to the finding in the analysis of the pooled data, a highly significant linear correlation between $[P_i]$ and $[\text{ADP}]$ was found over the studied range of steady-state power output levels ($r^2 = 0.98$; Fig. 5, inset). The variation of $[\text{ADP}]$ with power output for *subject A* is shown in Fig. 5. The shifted single substrate Michaelis-Menten rate equation (Eq. 11) adequately described the covariation (GFI = 0.43), similar to the finding for the pooled data. The estimates for C_1 , K_m^{ADP} , and C_2 were $134 \pm 16\%$ MVC, $22 \pm 16 \mu\text{M}$, and $42 \pm 25\%$ MVC, respectively (7 DF). Figure 6 shows the variation of $\ln([\text{ATP}]/[\text{ADP}][P_i])$ with muscle power output for *subject B*. Hysteresis was absent. The sigmoidal function given by Eq. 9 adequately described the covariation (dashed line; GFI = 0.48), although no significant estimate could be fitted for P_{max} . The estimates for P_{min} , n , and C were $15 \pm 10\%$ MVC, 0.4 ± 0.1 , and $12.5 \pm 0.4 \text{ M}^{-1}$, respectively (5 DF). The linear function given by Eq. 10 likewise adequately described the covariation of $\ln([\text{ATP}]/[\text{ADP}][P_i])$ and power output (solid line) ($r^2 = 0.93$; GFI = 0.43), although the agreement of the sigmoidal function appeared superior. The estimates for the slope and the

Fig. 4. Variation of adenine nucleotide concentrations (M) in fibers within superficial region of FDP, reflected in value of $\ln([\text{ATP}]/[\text{ADP}][P_i])$, with power output (%MVC) for an individual muscle (*subject A*). $\square$, Measurements at increasing power output levels performed in a ramp protocol; $\circ$, subsequent measurements at decreasing power output levels. Dashed and solid lines represent fit of Eqs. 9 and 10, respectively, to data. Regression equations are as follows: dashed line, $P = 66(e^{12.4 - \ln([\text{ATP}]/[\text{ADP}][P_i])} - 1) / (e^{12.4 - \ln([\text{ATP}]/[\text{ADP}][P_i])} + 3.6)$; solid line, $P = -16.2 \cdot \ln([\text{ATP}]/[\text{ADP}][P_i]) + 204$.

Fig. 5. Variation of ADP concentration (μM) in fibers within superficial region of FDP of subject A with power output (%MVC). Symbols are as in Fig. 4. Solid line represents fit of Eq. 11 to data. Regression equation is as follows: $P = 134/(1 + 22/[ADP]) - 42$. Inset: covariation of [ADP] and $[P_i]$ (μM and mM, respectively) in fibers over range of power output levels studied. Regression equation is as follows: $[P_i] = 0.25[ADP]$.

intercept were $-12.5 \pm 1.0\%$ MVC $\cdot$ M and $149 \pm 10\%$ MVC, respectively (7 DF). A linear covariation of $[P_i]$ and [ADP] over the studied range of power output levels was found for subject B ($r^2 = 0.93$) (data not shown). The shifted single substrate Michaelis-Menten rate equation (Eq. 11) adequately described the covariation of [ADP] and power output (GFI = 0.41) (data not shown). The fitted estimates for C_1 , K_m^{ADP} , and C_2 were $124 \pm 17\%$ MVC, $52 \pm 27 \mu\text{M}$, and $20 \pm 8\%$ MVC, respectively (6 DF).

The results of the fits of the various steady-state relations (Eqs. 9–11) to the pooled data of 20 subjects and 2 individual data sets are summarized in Table 2.

Fig. 6. Variation of adenine nucleotide concentrations (M) in fibers within superficial region of FDP, reflected in value of $\ln([ATP]/[ADP][P_i])$, with power output (%MVC) for a second individual muscle (subject B). Symbols are as in Fig. 4. Dashed and solid lines represent fit of Eqs. 9 and 10, respectively, to data. No estimate for P_{max} was obtained. Regression equations are as follows: dashed line, $P = P_{\text{max}}[e^{(5.0 - 0.4 \cdot \ln([ATP]/[ADP][P_i])} - 1)] / (e^{(5.0 - 0.4 \cdot \ln([ATP]/[ADP][P_i])} + P_{\text{max}}/15))$; solid line, $P = -12.5 \cdot \ln([ATP]/[ADP][P_i]) + 149$.

DISCUSSION

The reported findings of identical basal respiration rates of fast- and slow-twitch muscles with widely different basal ΔG_P values in the cat and mouse, respectively (9, 27, 28), suggest that the strictly linear description of the rate dependency of respiration on ΔG_P (7, 29) may not be quite appropriate in intact skeletal muscle. These findings are consistent with an alternative description predicting a sigmoidal relationship between the rate of oxidative phosphorylation and ΔG_P (28, 33, 40). We tested this alternative description using experimental data obtained from intact human forearm muscle using ^{31}P -MRS. We first discuss the methodological basis of our conclusions.

Methodology

Validation of negligible anaerobic ATP production. During exercise, the initial alkalization associated with consumption of hydrogen ions by net PCr hydrolysis was observed to reverse at the second work load, with pH maximally decreasing to a value of 6.90 at the highest power output level. This indicated a small glycolytic flux coupled to lactic acid formation superimposed on glycolysis coupled to oxidative phosphorylation, since glycolytic ATP production not coupled to substrate (NADH and pyruvate) formation for oxidative phosphorylation is accompanied by net production of H^+ , in contrast to oxidative ATP production (17). Some additional glycolytic ATP production could have occurred that remained undetected due to pH buffering by the cell or export from lactate from the cell. However, by virtue of the low yield of ATP per lactate compared with the yield of ATP per CO_2 , the contribution of ATP formed anaerobically to total cellular ATP production in the presence of oxidative phosphorylation would remain negligible.

Calculation of [ADP]. In previous studies of human forearm muscle (e.g., Refs. 1, 2), [ADP] was calculated from the creatine kinase equilibrium using the average values for [TCr] and [ATP] reported for human upper leg muscle (16). With the use of this approach, calculation of the average [ADP] in fibers of the FDP in the resting state for each subject yielded a wide range of values for [ADP] and $\ln([ATP]/[ADP][P_i])$ (Table 1). Because proton leak and adenine nucleotide concentrations dominantly control basal cell respiration (14, 15), this would suggest considerable intersubject variation in basal metabolic rate or mitochondrial function (uncoupling or slip of the proton pumps). With the assumption of similar mitochondrial function among all subjects studied, such magnitude of intersubject variation in basal metabolic rate would be inconsistent with reports of well-defined basal oxygen consumption rates in skeletal muscle (9, 19, 26). We therefore opted for an alternative approach to calculate [ADP]. We assumed identical values for [ATP] and $[PCr]/[TCr]$ in the resting state for all subjects. The physiological basis for the latter lies in the covariance of the $[PCr]/[TCr]$ and the phosphate potential for creatine-containing cells demonstrated independently by Meyer (29) and Connett (7).

Table 2. Values of the function parameters in Eqs. 9–11 for the fits to the pooled data of 20 subjects and 2 individual data sets

	$P_{\max}$, %MVC	$P_{\min}$, %MVC	C , M ⁻¹	n	m , %MVC·M	b , %MVC	C_1 , %MVC	C_2 , %MVC	K_m^{ADP} , μM
Pooled data	71 ± 18	18 ± 18	12.5 ± 0.1	0.8 ± 0.4	-14.9 ± 0.6	185 ± 6	120 ± 8	25 ± 5	37 ± 11
Subject A	66 ± 14	18 ± 30	12.4 ± 0.4	1.0 ± 0.7	-16.2 ± 1.6	204 ± 17	134 ± 16	42 ± 25	22 ± 16
Subject B	NS	15 ± 10	12.5 ± 0.4	0.4 ± 0.1	-12.5 ± 1.0	149 ± 10	124 ± 17	20 ± 8	52 ± 27

Values are means ± SE from regression. $P_{\max}$, $P_{\min}$, C , and n are function parameters of Eq. 9; m and b are function parameters of Eq. 10; C_1 , C_2 , and K_m^{ADP} are function parameters of Eq. 11. MVC, maximum voluntary contraction. NS, fitted estimate not significant.

With the use of this approach, a narrow range of values of [ADP] and $\ln([ATP]/[ADP][P_i])$ in the FDP in the resting state for 20 subjects was obtained (Table 1). We verified using data obtained from *subject A* that the pattern of variation of [ADP] and $\ln([ATP]/[ADP][P_i])$ with exercise did not depend on the approach used (data not shown).

Interference by microscopic heterogeneity within the FDP. In the analysis of the data, the FDP has been treated as a homogeneous muscle, both in the resting state as well as during exercise. However, the muscle is heterogeneous on a microscopic level, containing on average 47% type I and 53% type II (IIa + IIb) fibers (23). The difference in resting metabolic profile (e.g., P_i , PCr, and ATP content) between these fiber types in animal muscles (26, 27) remains, however, to be demonstrated for human muscle (36). Indeed, a ³¹P-MRS study of the individual variations in metabolic properties of human forearm flexor muscle showed no significant correlation between a putative in vivo measure of fiber type composition and resting PCr/ATP or P_i /ATP (3). However, recruitment of motor units during voluntary exercise resulting in subpopulations of active and inactive fibers within a muscle, with the number of active motor units increasing with power output (13), does complicate the interpretation of our results obtained during exercise. Two considerations were used in this. First, we addressed this issue using data obtained from *subject A*. For a number of hypothetical recruitment scenarios, we assessed the effect of the possible error in the estimation of the actual average PCr and P_i concentrations in contracting fibers from the measured overall PCr and P_i levels on the observed pattern of change in [ADP] and $[P_i]$ during exercise (data not shown). We concluded that recruitment-related microscopic heterogeneity within the sampled muscle during exercise would not interfere with the interpretation of the observed pattern of change in average [ADP] and $[P_i]$ and ΔG_P during exercise; the absolute values of the parameters obtained from their fits to the experimental data should, however, be interpreted with some caution. Second, based on a previously reported ³¹P-MRS study of human forearm muscle additionally employing electromyography in which it was found that recruitment of fast glycolytic fibers coincided with an abrupt decline in pH at pH ~ 6.90 (38), we considered it most likely that only motor units composed of oxidative fibers were recruited over the range of power output levels studied. As a result, the value of α in Eq. 2 would be similar for each subject in the population. Furthermore, the value

of one used in the modeling was of the correct order of magnitude, since there was agreement of the fitted value for $P_{\max}/\alpha$ with the experimental value of $P_{\max}$.

Variation of Adenine Nucleotide Concentrations With Power Output

In line with predictions of the behavior of the phosphate energy system in skeletal muscle cells during contractions at constant pH (7), the variation of adenine nucleotide and related metabolite concentrations during variation of muscle power output could be described by a single-variable function due to covariation of [ADP], $[P_i]$, [PCr], and [Cr] (see the APPENDIX; see also *inset* to Figs. 3 and 5). Accordingly, the variation of adenine nucleotide concentrations with power output in human forearm muscle could be adequately described by any of the mitochondrial driving functions derived by Connett (7), as demonstrated for the [ADP]- and $\ln([ATP]/[ADP][P_i])$ -dependent functions (Figs. 2 and 3 for the pooled data and Figs. 4 and 5 for the individual muscle data).

With regard to the nature of the function relating the steady-state rate of oxidative phosphorylation to its thermodynamic driving force ΔG_P over the range of physiological steady states in skeletal muscle, the agreement of a sigmoidal vs. a strictly linear relation with experimental data was evaluated on the basis of two criteria: the statistics of the fit of the respective functions to the data and the physiological relevance of each description. We emphasize that the sigmoidal description represents an extension rather than an antithesis of the linear description. Moreover, the linear description postulated in previous studies was based on a simulated relationship between ΔG_P and $(1 - [PCr]/[TCr])$, a putative linear measure of respiration rate at pH 7.0, that was sigmoidal over the full range of respiration rates (7, 29).

Statistically, the agreement with experimental data of the description predicting a sigmoidal relationship between the variation of ΔG_P with power output was equivalent to that of the strictly linear description. This was reflected in the similar values of GFI for the fit of Eqs. 9 and 10 to the pooled data (0.42 vs. 0.48, respectively) and to data of individual muscles (0.48 vs. 0.52, respectively, for *subject A*, and 0.48 vs. 0.43, respectively, for *subject B*). This finding can be attributed predominantly to the fact of an extensive quasi-linear domain in a sigmoidal function around its inflection point, reflected in the small error in the estimation of

the actual value of a sigmoidal function by the linear approximation of this domain (maximally 15% over 75% of the total range of values; Refs. 29, 40). The MNET description of oxidative phosphorylation is based on this actuality (40). Hence, to demonstrate statistically significant deviation from linearity, sampling of the sigmoidal function at its extreme values is required. In studies conducted on isolated mitochondria, full sigmoidal relationships between respiration rate and ΔG_P were indeed found, with measurements including the extreme respiration rates (states 4 and 3) (20, 28, 39, 40). Because the issue under investigation addresses a mitochondrial property, the same relationship should apply to intact cells. Indeed, we previously reported that the simulated relationship between ΔG_P and power output in skeletal muscle cells at constant pH 7.0 (i.e., in the absence of a cytosolic anaerobic glycolytic system) is sigmoidal (41). However, with respect to the experimental relationship in intact muscle, it is physiologically impossible to obtain measurements at the extreme respiration rates. The lowest measurable steady-state respiration rate corresponds to the basal ATPase rate rather than state 4, while the maximal measurable steady-state rate does not correspond to state 3 due to the activation of anaerobic glycogenolysis at high respiration rates (30). The limited sampling of this in vivo range of steady states feasible in the subjects due to the nature of the experiment further contributed to the finding of statistical equivalence of the agreement of the two models with the experimental data. Alternatively, this finding allows application of the linear MNET equations (40) to the study of skeletal muscle bioenergetics, offering a tool to address quantitatively the effect of changes in mitochondrial functionality (e.g., uncoupling or slip of proton pumps) or redox potential on the variation of adenine nucleotide concentrations with oxidative phosphorylation rate.

With respect to the physiological relevance of each description, we would suggest that the agreement of the description predicting a sigmoidal relationship between ΔG_P and power output with experimental data is superior to that of the strictly linear description based on three arguments. The first argument concerns the actuality that, as applies to all enzyme-catalyzed reactions, there is an upper and lower limit to the flux of oxidative phosphorylation (states 3 and 4, respectively, in classical isolated mitochondria terms). With respect to the former, there should thus be an upper limit to the maximal oxidatively sustained steady-state power output $P_{\max}$. This is predicted by the sigmoidal but not the strictly linear description.² Indeed, the sigmoidal description represents a hybrid of enzyme kinetics and thermodynamics in addition to an extension of the strictly linear description (28, 33, 40). As to the agreement with the experimental data, the estimate of this important func-

tion parameter from the fit of Eq. 9 to the pooled data (71% MVC) was in close agreement with the empirical value for the group of subjects studied (see METHODS). The highest measured power output sustained at constant pH 7.0 (± 0.1) in the population was 68% MVC. In comparison, the intercept of the linear fit to the pooled data was 185% MVC and was evidently of no relevance to $P_{\max}$. The same applied to the individual muscle data. For *subject A*, the empirical $P_{\max}$ was $\sim 64\%$ MVC, indicated by the observation of significant line broadening of P_i in the spectrum obtained at this power output level, whereas this was absent in all spectra obtained at lower power output levels. The fitted estimate of $P_{\max}$ for this subject (66% MVC) was in close agreement with this value, while the intercept of the linear fit to the data was 204% MVC. For *subject B*, the fit of the sigmoidal relation to the data did not provide an estimate of $P_{\max}$. We attributed this to the fact that the highest power output studied in this subject (52% MVC) did not approach the subject's $P_{\max}$; significant line broadening of P_i in the ^{31}P -NMR spectrum obtained at this power output level was still absent.

With respect to the lower limit to mitochondrial respiration, this actuality is reflected in the sigmoidal description in the parameter $P_{\min}$ in Eq. 9. Similar to the consideration of an upper limit, the strictly linear description does not predict a lower limit to respiration. With our experimental data, no accurate estimate for $P_{\min}$ was obtained, neither for the pooled data nor for the individual muscles (Table 2). The estimate for C , indicating the onset of the quasi-linear domain of the function ($\Delta G_P^\#$ in Eq. 4) equaled the value of $\ln([\text{ATP}]/[\text{ADP}][P_i])$ in the resting state in all cases (e.g., 12.5 vs. 12.5, respectively, for the pooled data). However, previously reported studies of intact rat (10) and feline (26) muscles do show that the relationship between steady-state submaximal oxygen consumption rate and ΔG_P is exponential (corresponding to the lower domain of the sigmoidal function) rather than linear, with the resting state explicitly in the lower nonlinear domain of the curve.

The second argument for superior physiological relevance of the sigmoidal description concerns the implication of a strictly linear relation between the rate of oxidative phosphorylation and ΔG_P . The latter predicts a constant sensitivity of respiration to phosphate potential changes over the full range of physiological steady states. In contrast, a sigmoidal relationship between the rate of oxidative phosphorylation and ΔG_P implies varying sensitivity to phosphate potential changes, reflected in the rate-dependent value of the slope ($dJ_P/d\Delta G_P$). According to the metabolic control theory (12) as well as results of metabolic control analysis of oxidative phosphorylation (14, 15), the predicted low sensitivity to phosphate potential changes at low respiration rates may be explained as reflecting shared control of the rate of respiration with proton leak, whereas the decreasing sensitivity at high rates reflects increasing sensitivity to redox potential (14, 15) as well as enzyme saturation effects.

² The slope and intercept of the linear function describing the covariation of ΔG_P and power output do bear a relation to the capacity for oxidation and phosphorylation (see Eq. 4). However, no direct estimate of this capacity can be inferred from the values of these function parameters.

The third argument is related to the previous one and pertains to the understanding of observations on intact skeletal muscle. The first of these regards the reported identical basal oxygen consumption rates for the feline biceps and soleus muscles and the mouse extensor digitorum longus and soleus muscles, while the basal phosphorylation potential differed between these fast- and slow-twitch muscles in each animal by as much as 3 kJ/mol (9, 26, 27). The strictly linear description of the rate dependence of respiration on ΔG_P cannot explain this observation in terms of the difference in mitochondrial density between fast- and slow-twitch muscle cells, because this by itself should lead to a very different sensitivity to changes in ΔG_P throughout the entire range of phosphorylation potentials. The study on intact feline muscles (26) showed this not to be the case; the sensitivity at basal respiration rate, i.e., the slope ($dJ_O/d\Delta G_P$), was similar for the fast-twitch biceps and slow-twitch soleus muscles. The low sensitivity to phosphate potential changes at low respiration rates predicted by the sigmoidal description offers an alternative explanation: the resting state ΔG_P values for the feline and mouse muscles mentioned above may reside on the virtually horizontal domain of the sigmoidal flow-force relationship. The second observation regards the onset of glycogenolysis at high respiration rates (30). The sigmoidal description predicts increasingly larger increments in [ADP] and $[P_i]$ for each step increment in oxidative phosphorylation rate at high respiration rates, thus providing a potential mechanism for the initiation of glycogenolysis activation (30). Moreover, the loss of correlation between respiration rate and phosphate potential at maximal rate predicted by the sigmoidal description provides a basis for understanding that the function of glycogenolysis activation at high respiration rates (and the concomitant steep decline in cellular pH) is to preserve cell function by preventing a collapse of ΔG_P .

In summary, the relevance of a sigmoidal description of the relationship between the steady-state rate of oxidative phosphorylation and the free energy of ATP hydrolysis in mammalian skeletal muscle is statistically equivalent yet physiologically superior to that of a linear description. We suggest to emphasize these issues by denoting the relationship quasi-linear rather than strictly linear. The upper limit of oxidatively sustained steady-state power output and the rate-dependent sensitivity of respiration to changes in ΔG_P predicted by the sigmoidal description are in agreement with both theoretical considerations of mitochondrial and mammalian skeletal muscle physiology, as well as experimental observations in the present study and other studies of intact mammalian skeletal muscle.

APPENDIX

Proportionality of ADP and P_i Concentrations

We define $[P_i]_0$ as the concentration of P_i remaining if at constant ATP and negligible ADP (and AMP) all creatine reacted to PCr through creatine kinase and H^+ -ATPase. Then

$$[PCr] = [TCr] - [P_i] + [P_i]_0 \quad (A1)$$

$$[Cr] = [P_i] - [P_i]_0 \quad (A2)$$

Through the creatine kinase equilibrium, we then obtain for [ADP]

$$[ADP] = \frac{[ATP]}{K'} \cdot \frac{[P_i] - [P_i]_0}{[TCr] - [P_i] + [P_i]_0} \quad (A3)$$

where $K' = K_{CK} \times 10^{-pH}$, where K_{CK} is the creatine kinase equilibrium constant. At constant [ATP] and pH, the first term of Eq. A3 is a constant. Whenever $[P_i]_0 < [P_i]$ and $[P_i] < [TCr]$, this relation predicts the ADP concentration to be proportional to the concentration of P_i . In case only the latter condition is met and $[P_i]_0 - [P_i] < [TCr]$, ADP is expected to vary linearly with P_i .

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