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Skeletal Muscle: A Paradigm for Testing Principles of Bioenergetics

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Muscular activity converts chemical energy into useful work and metabolism restores muscle to its original state. This essay explores the organization and interactions of the regulatory system(s) which allow this energy balance to occur. The term "energy balance" is used in a biochemical rather than a thermodynamic sense—concerned not with deductions from the physical principles of thermodynamics, but rather with those enzymatic processes which nature evolved and which operate at remarkably fixed stoichiometry. Energy balance is a statement of conservation of energy put into biochemical observables. ^{31}P NMR spectroscopy is one of the most useful techniques for investigating these questions quantitatively under physiological conditions *in vivo*. The author (1) describes the rules or principles of biochemical energy balance; (2) discusses sample results from human muscle to demonstrate its use in studying this class of questions; (3) presents a simple model of integrated cellular respiration to demonstrate its sufficiency to account for the main observations.

KEY WORDS: Energy balance; ^{31}P NMR spectroscopy; human muscle; cellular respiration; creatine kinase; feedback control; oxidative phosphorylation.

1. INTRODUCTION

Skeletal muscle contracts because an arriving nerve impulse releases acetylcholine to depolarize its membrane. Cardiac muscle contracts because pacemaker K^+ channels raise its membrane potential to threshold. In both cases, Ca^{2+} release initiates a large number of processes leading to chemical energy transformation. For human limb muscle at rest, the muscle's rate of energy utilization is low, on the order of 0.01 mM ATP $\cdot$ sec^{-1}. The rate of glycolytic ATP synthesis is negligible except under anoxia. The maximal rate of ATP generation is on the order of 0.4 mM $\cdot$ sec^{-1} but it is higher in leg muscle of highly endurance-trained individuals (e.g., cyclists capable of winning the *Tour de France*). Hence there is a ~40-fold range of ATP utilization and aerobic synthesis rates within which steady states of energy balance can occur in skeletal muscle. This metabolic scope is smaller in cardiac muscle because a baseline of a "heart at rest" is not a physiological state. The maximal rates are several times greater in heart than in skeletal muscle because of the larger amounts of mitochondria in the myocardium. Some muscle cells have a greater rate of ATP utilization than others. The differences are caused by isoforms of myosin, by Ca^{2+} pump ATPase, and by other cellular ATPases. It is possible that the ATPase rate can exceed the maximal capacity of oxidative phosphorylation, as is seen in some limb muscles, skeletal muscles designed for the highest mechanical power output. Here an energy balance is not possible, muscle activity "fatigues," and contractile activity must stop or decrease to allow the oxidative metabolism to catch up. Frog muscle is the best known example of this "twitch now, pay later" bioenergetic strategy which always is associated with delayed metabolism to restore the original resting state. The focus of this essay is energy balance, which can only be achieved if the magnitude of ATP generation equals the ATP utilization.

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2. BIOENERGETIC PRINCIPLES IN MUSCULAR ACTIVITY

The principles of biochemical energy balance are the products of evolution. They are summarized to yield a quantitative synthesis and model.

2.1. ATP Provides the Energy for All Forms of Muscle Work

Muscle is not a heat engine or fuel cell. Energy transformations only occur by chemical reactions. Lipmann used the phrase "high-energy phosphate bond" to point out that with all the intricacy of metabolic pathways, there is only a small set of common biochemicals involved in energy-transducing mechanisms, e.g., ATP (Lipmann, 1941). From the point of view of bioenergetics, the rest of the metabolic pathways define a biochemical anatomy which evolved to generate those "high-energy phosphate" molecules.

Muscular tissues, especially cardiac and skeletal muscle, are unique in that the rates of energy utilization in their cells can be varied over large ranges, and the transitions may occur rapidly (Kushmerick, 1983; Woledge *et al.*, 1986). Energy transductions in muscle are carried out by molecular electrochemical and chemo-osmotic transducing machines and chemomechanical motors. These synthesize or utilize ATP, the cell's source of chemical potential energy, in a way that couples this energy to the metabolic, electrical, osmotic, and mechanical work. Muscle is a chemomechanical system in which free energy of chemical reactions is coupled to mechanical performance by the actomyosin motor (Cooke, 1986; Brenner, 1990). Actomyosin interactions generating force and doing work and ion pumps doing electrical and osmotic work all rely on energy available from ATP, thereby coupling the exergonic process of ATP splitting to the endergonic processes of cellular work. Chemical input power must match the mechanical output power. The extent of energy dissipation uncoupled to ATP splitting is negligible. The exception may be seen at low ATP synthesis rates when leaks or slips of protons across the inner mitochondrial membrane dominate the mechanisms operating at low synthetic flux, but still the magnitude is small (Brand *et al.*, 1994). Muscles with different myosin isoforms have different costs for isometric force production and maintenance. Slow mouse skeletal muscle maintained the same force for about one-third of the ATP utilization rate as that of fast-twitch muscle (Crow and Kushmerick, 1982). In terms of actomyosin interactions, this result may be explained by a longer residence time of the attached, force-generating state of actomyosin motor in the slower muscle than in the faster.

2.2. Chemical Energy Is Stored in Cells—Concept of a Biochemical Capacitor

Certain forms of chemical potential energy are biochemically interconvertible by means of near-equilibrium reactions. The creatine-kinase reaction is perhaps the best known example of a bioenergetically important capacitance (Meyer *et al.*, 1984; Wallimann *et al.*, 1992) catalyzing the interconversion between ATP and phosphorylcreatine (PCr):

$$\text{ATP} + \text{Creatine} \longleftrightarrow \text{PCr} + \text{ADP} + \alpha\text{H}^+$$

The constant, α, is given to state that the stoichiometry is not necessarily unity, and depends on the pH at which the reaction occurs because the pK_a values of the reactants and products differ. The unique feature of muscle tissues is that transitions in their rates of energy utilization can occur rapidly, often more rapidly than the response times of ATP synthesis. Then the biochemical capacitance of PCr plays an important role; otherwise wide swings in the concentrations of ATP and ADP would occur. The content of PCr is effectively a capacitor of chemical energy by the nature of its dynamic near equilibration with its substrates and products (Meyer *et al.*, 1984). Muscle cells, and excitable cells in general, have significant concentrations of PCr higher than of ATP, as much as four times in skeletal muscle. Yet none of the chemical energy-transducing molecular machines is known to use PCr directly for coupling chemical energy to work production. PCr is a substrate for only one enzyme, creatine kinase (CK), but several isoforms of this enzyme are known and have been located in specific cellular sites (Wallimann *et al.*, 1992). As a capacitor, PCr represents chemical potential energy previously generated by oxidative metabolism. Thus, endergonic processes can be temporarily coupled to free-energy dissipation without the requirement for simultaneous oxidative metabolism provided the limits of the chemical energy capacitance, the supply of PCr, are not exceeded. Of course less free energy is dissipated the closer the CK reaction is to equilibrium, and the concept of chemical capacitance depends on near equilibrium. The evidence

shows the CK reaction operates near equilibrium in muscle cells (Meyer *et al.,* 1984; McFarland *et al.,* 1994; Ugurbil *et al.,* 1987). Nonetheless, it must be true, especially during high flux, that the reaction departs from true equilibration, but is it not known experimentally just how close to (or far from) equilibrium the CK reaction remains during rapid transitions from rest to work, and vice versa.

Creatine kinase exists in muscle cells as several isoforms, including one (MM-CK) known to bind to the M-line of myofibrils and distributed in the cytoplasm and another (Mi-CK) located on the inner side of the outer mitochondrial membrane (Wallimann *et al.,* 1992; Wyss *et al.,* 1992). In skeletal muscles about 90% of CK is the MM-CK isoform. It is clear that complex interactions occur within the intermembrane space between the substrate and products of the CK reaction and transport from this space to the mitochondrial matrix space and to the cytoplasm (Wallimann *et al.,* 1992). Understanding the function of the localized CK isoforms remains incomplete. Nonetheless, evidence is quite clear that the bidirectional fluxes catalyzed by cytoplasmic CK are sufficiently large that the net PCr splitting caused by contractile activity and its resynthesis during recovery do not perturb this reaction very far from equilibration when the cell as a whole is considered (Meyer *et al.,* 1984). The small distances between sites of PCr and ATP utilization and resynthesis can be explained by simple diffusion (Yoshizaki *et al.,* 1982, 1987, 1990). ATP and ADP are free to diffuse and share in this coupling, even though most of the metabolite flow occurs as PCr and Cr (because they are present in higher concentrations than ATP and ADP, respectively [Meyer *et al.,* 1984]). Even in cardiac myocytes which may have mitochondrial volume densities of 35% of total cell volume in rat cells, most of the substrate is in the cytoplasm and near equilibration is observed (Kingsley-Hickman *et al.,* 1987). The details of CK function in skeletal muscle can be understood in terms of this simple model of CK function (McFarland *et al.,* 1994). In that work, the flux *in vivo* was lower than expected from *in vitro* measures of its activity, and required the presence of nonreactive complexes (PCr·ATP·enzyme and Cr·ADP·enzyme, the dead-end complexes) to account quantitatively for the results. So complexities remain. The deletion of MM-CK in a transgenic mouse (Van Deursen *et al.,* 1993) led to apparently normal development, including cardiac and skeletal muscle. But it was clear that functional differences could be detected (Van Deursen *et al.,* 1994). The experiments demonstrate that the total amount of CK in a muscle cell and its cellular distribution are important aspects of CK function. Perhaps the most difficult result not yet explained are the discrepancies between the CK activity assayed by ^{31}P NMR spin-transfer methods and by *in vitro* biochemical assays (Van Deursen *et al.,* 1994). Whereas the latter showed that a graded expression of MM-CK isoform was achieved genetically, the NMR assay showed a highly nonlinear relationship, and did not detect expression at even 1/3 the normal expression; the method used should have been sensitive to detect that activity. The conclusion must be either that the mitochondrial form of the enzyme can substitute for the cytoplasmic isoform but not for all aspects when quantitative measures are compared, or that cytoplasmic MM-CK is not relevant to muscle bioenergetics. The expression of the BB isoform of CK in liver of transgenic mice (Miller *et al.,* 1993) gave results consistent with energy buffering as expected from classical function of CK in muscle, so it seems clear that when present in the cytoplasm of cells CK functions can be understood as a buffer and capacitor. Thus, the kinetics and fluxes in tissues containing MM-CK or other cytoplasmic isoforms will be dominated by the metabolites in the cytoplasmic space and the action of the cytoplasmic isoforms because the size of the metabolite pool in the mitochondria is small. So the concept of PCr as a biochemical capacitor remains valid, even though there are aspects of CK function that are not completely understood. Certainly a more complete quantitative model of high-energy phosphate fluxes in a muscle cell will be needed to account for these recent results than is presented in the last section of this essay; however, we will see that the present principles and simple models are sufficient to account for the main features of muscle bioenergetics.

2.3. The Sum of the Coupled ATPases Sets the Demand Side of the Balance and Defines Energetic States

Skeletal muscle can be considered "the servant of the nervous system" in that activation of the ATP-utilizing machines originates in physiological controls outside the muscle cell (Burke and Edgerton, 1975). These mechanisms produce an increase in calcium ion concentration which removes troponin-tropomyosin inhibition of the actomyosin motor. We have already emphasized that a unique feature of striated muscle is that the bioenergetic scope is large compared to other

cell types. This cascade of events allows ATP hydrolysis coupled to cellular work output. Otherwise the ATPase rate is extremely low, on the order of 1 or 2 orders of magnitude lower than the maximal. The magnitude of ATP hydrolysis uncoupled to the molecular transducers (in analogy to the proton leak dissipating chemical energy in the mitochondria without coupling to ATP synthesis) is not precisely known, and, on the basis of the very low rate of metabolism in muscles at rest, is thought to be very small. Calcium release also induces its own energy-using transport back to the sarcoplasmic reticulum (Kushmerick, 1983; Woledge *et al.,* 1986). The total energy required for all of these processes is then equal to the sum of all the ATP-utilizing mechanisms. In order of magnitude the most important ones are: actomyosin ATPase > Ca^{2+}-activated transport in the sarcoplasmic reticulum >> other ion pumps >> macromolecular biosynthesis. The sum of all of these sets the energy demand side of energy balance.

The rate of the free-energy-driven mechanisms is the primary and causal mechanism in muscle bioenergetics. The overall metabolic rate of a cell changes because its ATPase rates change. The converse is also true: if a measure of the overall metabolic rate increases, then the steady-state sum of the ATPases have increased. In terms of the concepts of metabolic control analysis, the control strength of the calcium-activated ATPases initiated by the onset of muscular activity is unity, and this flux controls the flux of oxidative phosphorylation. While this statement might appear obvious, this proposition has never been formulated into an appropriate metabolic control analysis model. If this scheme is valid for all physiological conditions, neither the availability of extra oxygen or substrate *per se* nor an increase in the magnitude of the chemical potential stored in ATP and PCr concentrations will increase the metabolic rate. Only a change in the rate of the ATPases does that.

2.4. The Products of ATPases Provide Control Signals for Energy Balance

Cells have a remarkable constancy of the concentration of their constituent metabolites despite widely varying rates of function. "Homeostasis" is a commonly used term to describe the various states of living systems. A much more appropriate term is "homeodynamics" which connotes complex interacting kinetic systems operating at one of a number of possible steady states to achieve energy balance. How is this constancy achieved in the face of varied levels of demand? It is useful to distinguish two broad classes of regulatory mechanisms: feedback and feedforward.

In feedforward regulation, the signals which activate the ATP-utilizing mechanisms act in parallel to provide information to the processes synthesizing ATP. In that sense, the ATPases and ATP synthesis are not mechanistically coupled, although they are correlated. These normally balanced molecular processes activated by a common external signal could result in energy balance only if the results of their activities are exactly matched. This mechanism is essentially the role envisioned for calcium activation of mitochondrial dehydrogenases (McCormack and Denton, 1990, 1993). Feedforward mechanisms involve alterations of enzyme activities or substrate affinities, such that apparent kinetic constants, maximal velocities, or coefficients of thermodynamic relationships are regulated variables. The crucial corollary of this class of mechanisms for energy balance is that their operation must exactly match ATP synthesis to ATP demand if regulation is to be effective. The gain of the responses of each component of energy balance to a given Ca^{2+} signal is an example of one possible feedforward signal, e.g., it must be exquisitely matched so that the ATP synthesis equals the ATPase quantitatively. Otherwise PCr and ATP would build up in the cell over time (or be depleted) independent of muscle activity.

Therefore the argument is compelling that a signal (or signals) derived from the coupled chemo-mechanical machine must be present and functional as a feedback regulator of metabolic ATP synthesis irrespective of the existence of such feedforward mechanisms. The results obtained from animal and human skeletal muscle are quantitatively consistent with a feedback model (Meyer, 1989; Kushmerick, 1981; Blei *et al.,* 1993a) and that model is sufficient for the present precision of the data. For this to occur, the signal molecules must be one or more of the products of ATP-utilizing molecular machines: inorganic phosphate (Pi), ADP, H^{+}, or creatine (from the capacitance in creatine kinase). Internal signals regulating intracellular ATP synthesis is the essential feature of feedback signals. Feedback control predicts that cellular concentration of some or all products of ATP splitting are error signals and play important roles in regulating ATP synthesis. If the result of the feedforward signals were in the slightest way imbalanced, the operation of feedback control becomes necessary, and may be sufficient, to restore energy balance. The presence of feedback

control does not of course negate the existence of feedforward ones, nor does the apparent absence of pure feedback regulation, known under some conditions in cardiac muscle, mean that feedback regulation by products of ATPase are not important mechanistically.

Many papers in the past several years have attempted to identify specific mechanisms of the regulation of oxidative phosphorylation in various types of muscle. Typically only a relatively small range of the full physiological scope is explored. Cellular respiration is subject to multiple regulatory mechanisms (Groen *et al.*, 1982), and the control strength of specific components of the mitochondria depends on where the mitochondria are functioning on the scale from minimal to maximal rates of flux. Cellular respiration is a system property of the cell, not a single mechanism. Only when a full set of measurements of most or all of the component variables can be experimentally verified and then over the full range of fluxes will one arrive at an "understanding" of cellular respiration regulation. Some of the evidence indicating that cardiac muscle does not use cytoplasmic feedback signals may be an artifact of the small range of experimental manipulation achieved compared to the full range possible in normal physiological condition. Experimentally, it is much easier to achieve a very wide range of metabolic activities in skeletal muscle than in cardiac muscle, for example. If one compares the range of myocardial oxygen consumption measured in exercising dogs (Huang and Feigl, 1988) with that used experimentally for studies of regulatory signals (Katz *et al.*, 1989; Heineman and Balaban, 1990), one finds that the latter studies usually achieved much less than one-half of the full metabolic scope of which those hearts are capable.

2.5. Muscles, Which May Differ Quantitatively in the Magnitude of Their ATPase and Synthesis Capacities, Achieve Energy Balance but with Different Metabolite Concentrations and Biochemical Steady States

If the cell uses all of its stored energy and if ATP synthesis cannot maintain energy balance, its function fails. We commonly refer to this state as "fatigue" which occurs where some (or all) of the feedback signals are inhibitory for ATP-utilizing mechanisms (Fitts, 1994). ATP-synthesis balancing ATP is then separable in time. A well-known example of this is a maximally activated fast-twitch muscle of the frog cited earlier. Consider the opposite case in which size of the maximal ATP synthesis can greatly exceed the magnitude of the ATPases. Then observations on cellular respiration will appear as if there is little or no feedback error signal operating. Such results could lead to the erroneous conclusion that feedback mechanisms do not exist. The size of the error signal for feedback control in this example will be small, perhaps even within the noise of the experiment. Thus, for the same absolute amount of energy utilization, energy balance is obtained with different biochemical states, depending upon the metabolic repertoire of the muscle cells. Larger changes in PCr and ATP will occur in mitochondrial-poor muscle to achieve a given absolute rate of oxidative phosphorylation. The reason is simply that for a given enzyme activity, the error signal in the feedback control (i.e., the concentration of the signaling metabolites) will be larger for lower mitochondrial contents than for larger contents to achieve the same flux. This effect is easiest to visualize for a simple Michaelis–Menten formulation. Variable fluxes for the same V_{max} require different substrate concentrations; cells with different oxidative phosphorylation activities (i.e., different V_{max}) will achieve the same flux with different substrate concentrations. The effective "gain" in the system for cellular respiration is a property of the cellular enzyme activity, and, as in the myocardium compared to skeletal muscle (~30% by volume mitochondria versus ~ 6%), will be very large. Small signals will produce big effects. This effect was precisely what was observed in rat skeletal muscles in which the mitochondrial enzyme activity was varied as a function of thyroid state (Dudley *et al.*, 1987).

It is well known that muscles differ in their mechanical and metabolic kinetics, and in their sustainable mechanical and metabolic power (Pette and Staron, 1990). Isoforms exist for most if not all of the components: in the actomyosin machine, in mitochondria, in the calcium transport in the sarcoplasmic reticulum, and in sarcolemmal ion channels. Thus, differences in the constituent proteins provide for a range of functional power for both ATP use and synthesis. A mechanically fast muscle has a greater rate of coupled ATPase than a slower muscle. Barany's classical correlation of actomyosin ATPase with maximal shortening velocity (Barany, 1967) suggests myosin determines the mechanical and chemical V_{max}. The molecular mechanism is not clearly settled. The separate roles for the myosin heavy chain (known to be

important) and light chains have not been identified completely (Eddinger and Moss, 1987; Larsson and Moss, 1993), nor is the role played by activation mechanisms on the thin filament in the regulation of actomyosin kinetics (Moss, 1992; Chase *et al.*, 1994). Evidence indicates that a great number of permutations and combinations of isoforms can exist, so that there is a continuum of mechanical properties in a skewed gaussian distribution (Pette and Staron, 1990; Nemeth *et al.*, 1979). Similarly, enzyme analyses of single cells show a continuum of activities. Muscles also differ in their mitochondrial content such that their maximal rates of substrate oxidation and oxidative phosphorylation differ. While we have come to recognize these muscle characteristics as stereotypes (Saltin *et al.*, 1983), e.g., fast-twitch glycolytic or slow oxidative muscle types, the stereotypic vision can be misleading. Instead of a distribution of various fractions of narrowly defined cell properties, the evidence in human muscle for a continuum of fiber mechanical and metabolic properties means that a macroscopic observation of a whole muscle or group of muscles yields information on the median (or some other average) of the property measured. Clear examples of biomodal distributions are relatively rare (Mizuno *et al.*, 1994). In specific muscles of individual human subjects there is typically no more than a twofold difference in average properties. Examples of a greater range have been described when stereotypic examples are sought in various mammalian muscles (Kushmerick *et al.*, 1992a); the range is larger in amphibians. In human subjects, where individual subjects have been described (as opposed to group averages), it is clear that the physiological and bioenergetic interpretations can be made as a population distribution. We will revisit this question in Section 3.3.

3. EXPERIMENTAL OBSERVATIONS ON HUMAN MUSCLE

3.1. Separability of ATP Utilization from ATP Synthesis in Electrically Stimulated Muscle

In all studies of normal voluntary muscle activity, the ATP utilization and resynthesis are usually occurring simultaneously due to the dynamic processes of energy balance. The use of ischemia allows aerobic ATP resynthesis to be delayed until restoration of blood flow (Blei *et al.*, 1993a); under the conditions of our initial experiments, there was negligible anaerobic glycolysis. The basic experiment and results are displayed in Fig. 1. PCr changes relative to the sum PCr and Pi are plotted as a function of time during the whole experiment because the stoichiometric relationship, $\Delta PCr = -\Delta Pi$, was found for PCr and Pi changes. The PCr breakdown rate during ischemia before stimulation is not readily visible in the figure; it was measured independently and provided a measure of the basal ATPase rate (0.008 mM PCr per sec), a value close to that found by muscle biopsy (0.01 mM/sec) (Harris *et al.*, 1975). The linear PCr breakdown during stimulation represents the ATP use of the summed ATPases of the muscle with only a small underestimation due to the glycolytic ATP production. The mean calculated ATPase rate for our eight subjects at 1 Hz was 0.15 mM per sec, which is approximately a fifth of the maximal ATPase activity estimated for human forelimb muscle [*in vitro,* 0.75 mM/sec (Barany, 1967); *in vivo,* 0.5–1.0 mM/sec (Boska, 1991)]. Thus, for the range of stimulations used in our experiments, the forearm musculature operated far from its maximal ATPase rate as expected for low-frequency twitch-stimulation resulting in unfused mechanical responses. Twitch stimulation at 1 Hz is a relatively small metabolic stress but still produced an 18-fold (0.15/0.008 mM PCr per sec) metabolic scope.

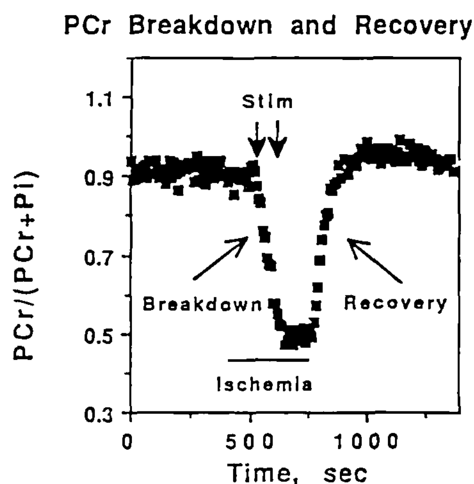

Fig. 1. The time course of PCr changes during an ischemic stimulation protocol of the human forearm flexor muscles with subsequent oxidative recovery with normal blood flow is shown. Total duration of the experimental time is given on the abscissa. The horizontal bar indicated the time of ischemia produced by inflation of a blood pressure cuff above systolic pressure. The PCr change is normalized to the total content of PCr and Pi at each time point; time resolution is 9 sec. Data were obtained by ^{31}P NMR spectroscopy. (Reproduced from Blei *et al.*, 1993b, which can be consulted for full details.)

Release of the pressure cuff allowed aerobic recovery by restoring blood flow and oxygen delivery with initiation of mitochondrial oxidative phosphorylation. Delivery of oxygen to the mitochondria from the restored circulation required about 10 sec. PCr content return to the resting, prestimulation level and the time course was monoexponential (Blei *et al.*, 1993a; Meyer, 1988). Recovery kinetics of PCr following exercise are in accord with a simple model of respiratory control (Chance and Williams, 1956; Chance *et al.*, 1986) or with the model of linear thermodynamic control of mitochondrial respiration (Meyer, 1988). Neither can be distinguished from a sigmoidal curve predicted by nonequilibrium thermodynamics (Westerhoff and van Dam, 1992) in these types of experiments. These results indicate that our protocol provides a measure of a muscle's functional properties that reflect the underlying oxidative and contractile ATPase activities. Classic cell typing similarly characterizes muscles in terms of the oxidative and contractile ATPase capacities with high-economy, slow-twitch cells having low ATPase rates and greater ATP-synthesis rates than lower economy fast-twitch cells. Our protocol also separates the contractile from oxidative properties using dynamic, graded, quantitative, and noninvasive measures: the economy of muscles is revealed by the cost-per-twitch, while the recovery phosphorylation rate provides a means of estimating oxidative capacity. Thus, our measurements of ATP breakdown and recovery rates provide the same information sought by muscle cell typing.

3.2. Individual Differences Are Meaningful Mechanistically

Three features of our protocol were designed so that functional measurements are made under the same physiologic conditions in each subject. First, percutaneous stimulation of the motor nerve activates the entire muscle so that all muscle cells in the region of interest were fully activated with each nerve activation. All of the cells are recruited simultaneously with each maximal twitch. The primary remaining variable influencing energy utilization per twitch is the variation in characteristics of the individual cells. Anaerobic PCr breakdown measures the cost-per-twitch for each subject. Thus, with the intracellular H^+ and ATP levels not significantly altered, the rate of recovery should be reproducible and faster in those muscles with a greater oxidative capacity. With these precautions, individual differences in both components of energy balance were measured (Blei *et al.*, 1993b). These properties are important in defining the contractile and metabolic properties of muscle because cells differ in their cost-per-twitch and mitochondrial contents. Separation of PCr breakdown and recovery provides functional measures of the underlying myosin isoform (cell-type) composition and mitochondrial content of the tested muscle. Figure 2 displays for the individuals identified by single letters these components of energy balance. Three repeat measures for each individual had a relative standard deviation of about 10%. Panels A and C of Fig. 2 show the largest and smallest of the rates observed. Panels B and D display the results of each individual in rank order. The significant difference in the 1.6-fold range of values indicated that our measure of muscle cost-per-twitch is sensitive enough to distinguish individuals. The cost-per-twitch was negatively correlated with Pi/ATP. Thus, the muscles with the highest cost-per-twitch have the low Pi/ATP indicative of a greater proportion of faster-twitch cells, while the lowest ~P cost-per-twitch is found in individuals with the high Pi/ATP indicative of a slower-twitch muscle (Conley, 1994). This correlation in contractile economy is consistent with results from animal muscle, where there are much stronger correlations between concentrations of PCr, ATP, Pi and the chemical potential of ATP between cells of different types (Kushmerick *et al.*, 1992a, b). Thus, the individual differences in ~P cost-per-twitch corresponded with differences in phosphorus metabolite content indicative of cell type in mammalian muscles with both measures of energy balance providing an indication of the cellular characteristics of a muscle. ATP cost-per-twitch activation provides a functional measure of the isoform content and cell type composition of human muscle. Individual recovery time constants differed by 1.8-fold and thus reflect the oxidative capacity of the muscle.

Because our results show that both the capacity for ATP breakdown and recovery can vary by nearly twofold among subjects, the balance of ATP supply and demand represented by the PCr/Pi level can be achieved by a number of combinations of breakdown and resynthesis capacities. Steady-state PCr level during exercise, then, does not uniquely characterize muscle properties. Both components of energy balance are needed for the serial monitoring of muscle properties through exercise training, therapeutic interventions, or disease states.

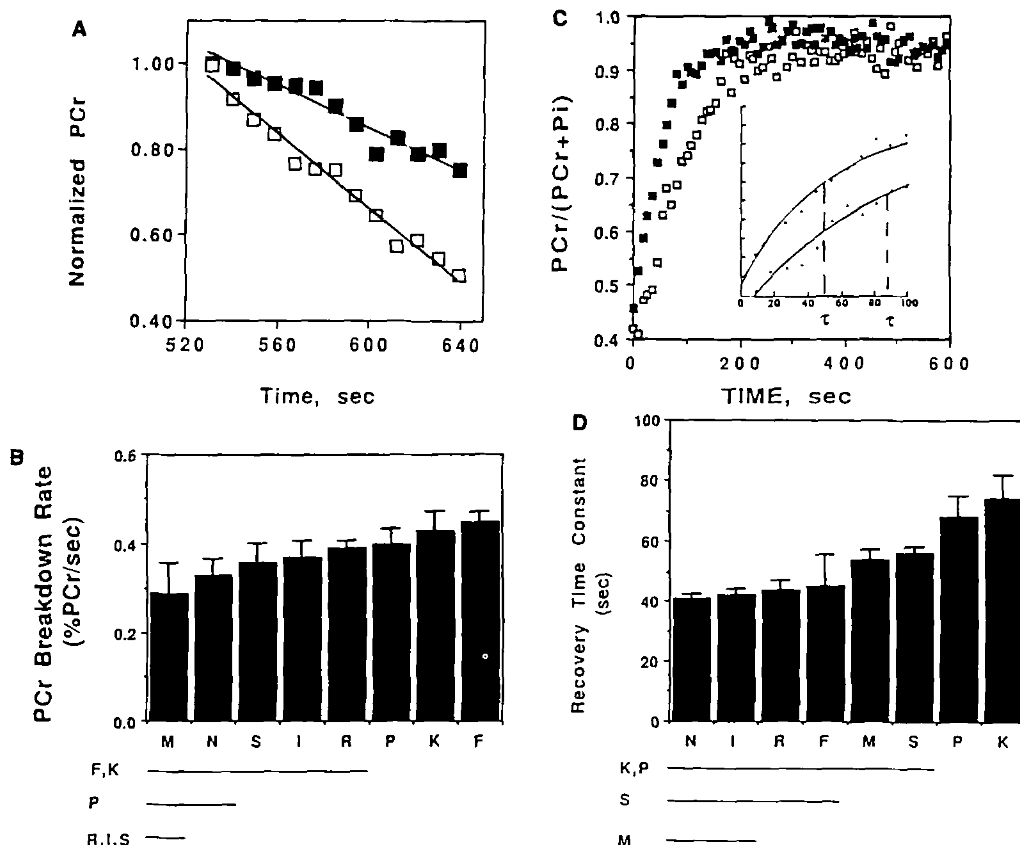

Fig. 2. The range of individual differences (rate of energy use and synthesis) found with the experimental protocol in Fig. 1 is displayed. Each panel represents data obtained by triplicate observations of single individuals. Panel A displays a portion of the data obtained during ischemic stimulation of two individuals. The slopes shown were the highest and lowest rates of PCr decrease observed. Panel B displays the mean ($\pm$ one S.D.) of the PCr utilization rate for each subject. Horizontal lines indicate individuals significantly different from the others. Panel C displays the recovery portion of the data obtained in two individuals with the largest and smallest time constant for oxidative recovery. Panel D displays the mean ($\pm$ one S.D.) of the recovery time constants for each subject. Data were obtained by ^{31}P NMR spectroscopy. (Reproduced from Blei *et al.*, 1993b, which can be consulted for full details.)

3.3. The Issue of Cellular Heterogeneity

If there are distributions of enzyme activities and substrate and product concentrations in the various cells, and the metabolic rates are non-linear functions of those quantities, then the biochemical interpretation of observations of populations of cells may be complex, difficult, or even wrong. The question here is: What is the magnitude of these differences in human muscle? My argument will be that regions of human muscle amenable to study by NMR and biopsy techniques can be considered in many subjects to be gaussian or skewed gaussian distributions; it is possible but less frequent to find clear evidence for biomodal distributions (Mizuno *et al.*, 1994). For our purposes, possible heterogeneity effects can be ignored in the macroscopic measurements provided that due attention is paid to anatomical differences (Jeneson *et al.*, 1992) and care is given to identify the physiological state of the muscle, as was done in our experiments by activating all of the muscle cells by direct neural stimulation. This control and knowledge of muscle activation is not generally known in graded voluntary muscle activity for two reasons. First, different anatomical regions may be used as the intensity of the activity changes during the voluntary task. Secondly, the spinal and supra-spinal levels of physiological control of the motor units are recruited in order of nerve and muscle cell size (Henneman *et al.*, 1965a,b; Burke *et al.*, 1973). These functional differences between the most easily

activated cells and those requiring the greatest neural intensity select different population of cells depending on the intensity of the voluntary activity.

Properties of individual muscles cells can be distinguished and classified on the basis of a number of anatomical, physiological and biochemical criteria into various categories of cells called fiber types (Saltin *et al.*, 1983). Different classification schemes result depending on the criteria chosen because each characteristic distinguished defines only some aspects of muscle structure and function. A single criterion does not necessarily generate the same category of "fiber types" as another. There is a continuum of cells and their properties (Pette and Staron, 1993). Muscle cells also differ in the concentrations of the metabolites involved in energy metabolism. Measurements of metabolite composition demonstrated a higher PCr and lower Pi contents in the fast-twitch muscle compared with a slow-twitch muscle (Crow and Kushmerick, 1982; Meyer *et al.*, 1985; Kushmerick *et al.*, 1993). However, these easily detectable differences in selected animal muscles have been hard to detect in human muscle. ^{31}P NMR data indicate a slightly higher Pi and lower PCr content in the soleus than in the gastrocnemius by the use of localized spectroscopic techniques to sample regions totally within each muscle (Vandenborne *et al.*, 1993). These ^{31}P NMR data confirm analyses of single fibers from human muscle, sorted histochemically, which showed only small differences in PCr contents between types 1 and 2 fibers (Edstrom *et al.*, 1982; Greenhaff *et al.*, 1993; Söderlund and Hultman, 1991), and no differences in the ATP content. The conclusion must be that in a given human muscle the range of differences in metabolite composition is smaller than what is predicted from the examples from laboratory animals, and this narrower range is due to a narrower distribution of cell properties. Although the total creatine, PCr, and ATP concentrations can differ, the ratio of PCr/ATP is relatively constant in animal muscle cells (Conley, 1994; Connett, 1988) and more so in human muscles. The implication of this constancy is that cells have a similar ADP concentration and chemical potential of ATP at rest. Likewise the relative changes in metabolites will be similar at any fraction of the full range of activity of oxidative phosphorylation; for example, this scheme suggests that similar cellular composition will be found in muscle cells operating at any fraction of their full aerobic metabolic scope. If mitochondria in all muscle cells have the same properties quantitatively (as evidence suggests), then this sort of relative scaling should allow fast-twitch muscle, slow-twitch muscle, and perhaps even cardiac muscle to be superimposable on a common scale when oxidative phosphorylation flux is plotted as an appropriate function of metabolites.

The mitochondrial volume density is a measure of the capacity for oxidative phosphorylation (Hoppeler, 1986). By this criterion, single cells from human vastus lateralis show a wide overlap (Howald *et al.*, 1985). The type of myosin isoform present determines the mechanical speed of shortening and the ATPase activity (Barany, 1967). By this criterion, single human muscle cells show as great as a 10-fold range of maximal velocities (Larsson and Moss, 1993). When these distribution are scaled to the cell types present in the human forearm, not more than a 3-fold range of speed and ATPase activity is expected. We showed important individual differences did exist in the observed ATPase and in the ATP synthesis rates in human muscle, and that these covered a range of about 2-fold (Blei *et al.*, 1993b). So the conclusion is that despite distinctions which can be made, observations in given human muscle indicate that in an individual muscle the functional heterogeneity is on the order of experimental error. The rate of PCr breakdown and resynthesis following three identical activations of the forearm musculature repeated in the same individual was highly repeatable (approximately ± 10%), but the individuals differed among themselves by almost 200% (Blei *et al.*, 1993b). Also individuals can be grouped on the basis of the kinetics of recovery of PCr following graded voluntary exercise (Mizuno *et al.*, 1994) and show recovery

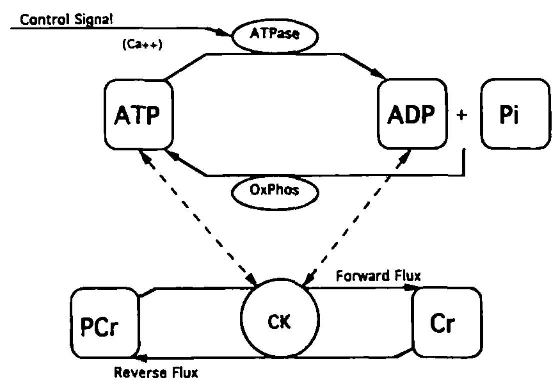

Fig. 3. Schematic of a bioenergetic model describing the events of a contraction and recovery cycle of muscle. The first element is the summed ATPase controlled by calcium ion concentration. The second is a closed loop feedback regulation of oxidative phosphorylation by the products of the ATPase activity, ATP and Pi. The third is the cytosolic CK reaction in which the forward and reverse fluxes are functions only of the concentrations of its substrates and products.

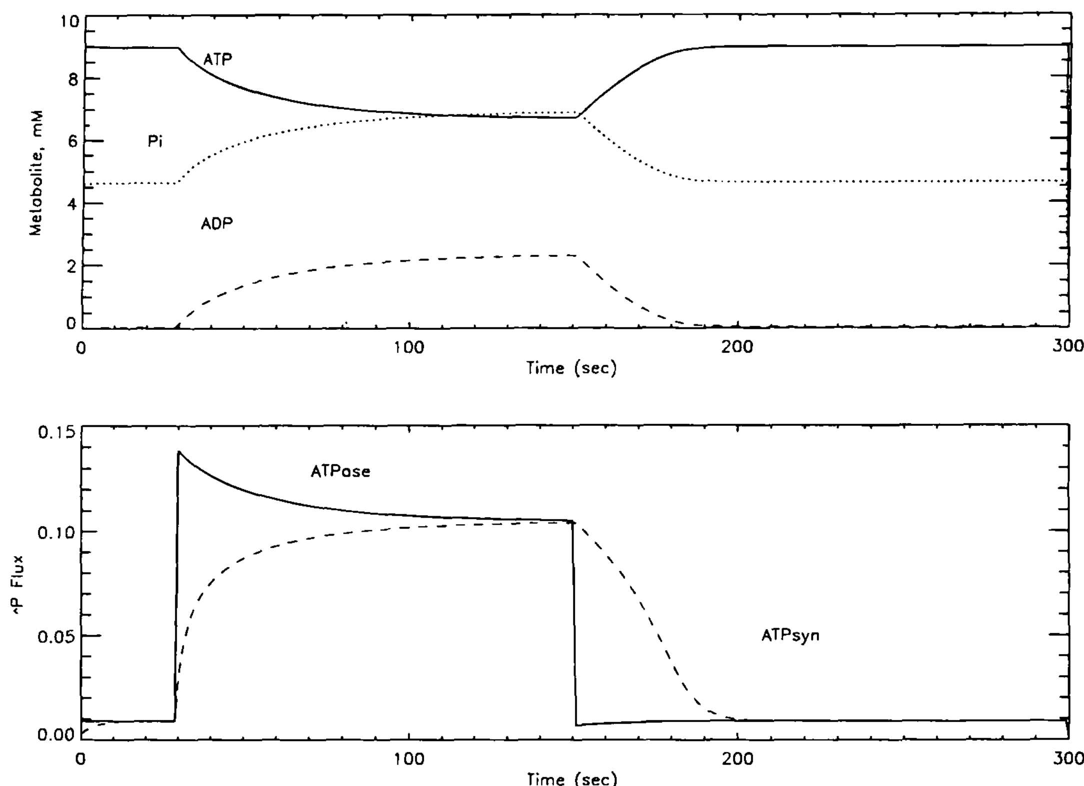

Fig. 4. The results of computer simulations based on the ATPase and oxidative phosphorylation components only of the model shown in Fig. 3.; these simulations did not include CK function. Panel A displays the result when ATPase was increased with concomitant operation of oxidative phosphorylation. It thus simulates feedback regulation of calcium-activated ATPases coupled to oxidative phosphorylation as described in the text. The coupled system approaches a steady state within 120 sec for the conditions used. Panel B displays the result when oxidative phosphorylation is inactive until the time indicated (150 sec), and represents a simulation of ischemic stimulation followed by aerobic recovery.

kinetics that were explainable on the basis of the histochemical cell types of biopsies from the same region sampled by the NMR experiment. These results all show that the inter-individual variation in energy metabolism is related closely to cellular composition of the specific muscle studied. The conclusion is that any given human muscle can be best represented as a unimodal continuum of properties. Therefore macroscopic measurements of cellular bioenergetics can be reliably interpreted in terms of intracellular and molecular mechanisms. Individuals differ because their component cells differ. This conclusion has the important corollary that when possible, hypotheses concerning bioenergetics, energy balance, and regulation should be tested on single individuals for the greatest precision and least ambiguity.

4. QUANTITATIVE MODEL FOR MUSCLE BIOENERGETICS

It is straightforward to translate the principles discussed in Section 2 into a simple model of muscle bioenergetics. Such a scheme is given in Fig. 3. ATPase is controlled by calcium ion as a switch between a low ATPase rate constant at rest to a higher rate constant for contraction. For simplicity only two states are considered, so the ATPase flux = $k_{rest} \cdot$ [ATP] or $k_{contract} \cdot$ [ATP]. Glycolysis and changes in pH are omitted for simplicity, and will be required for a more faithful model. ATP, Pi, and ADP are the shared metabolites for ATPase and for oxidative phosphorylation. Because all control of flux is by definition in the ATPase by calcium, the flux of oxidative phosphorylation depends

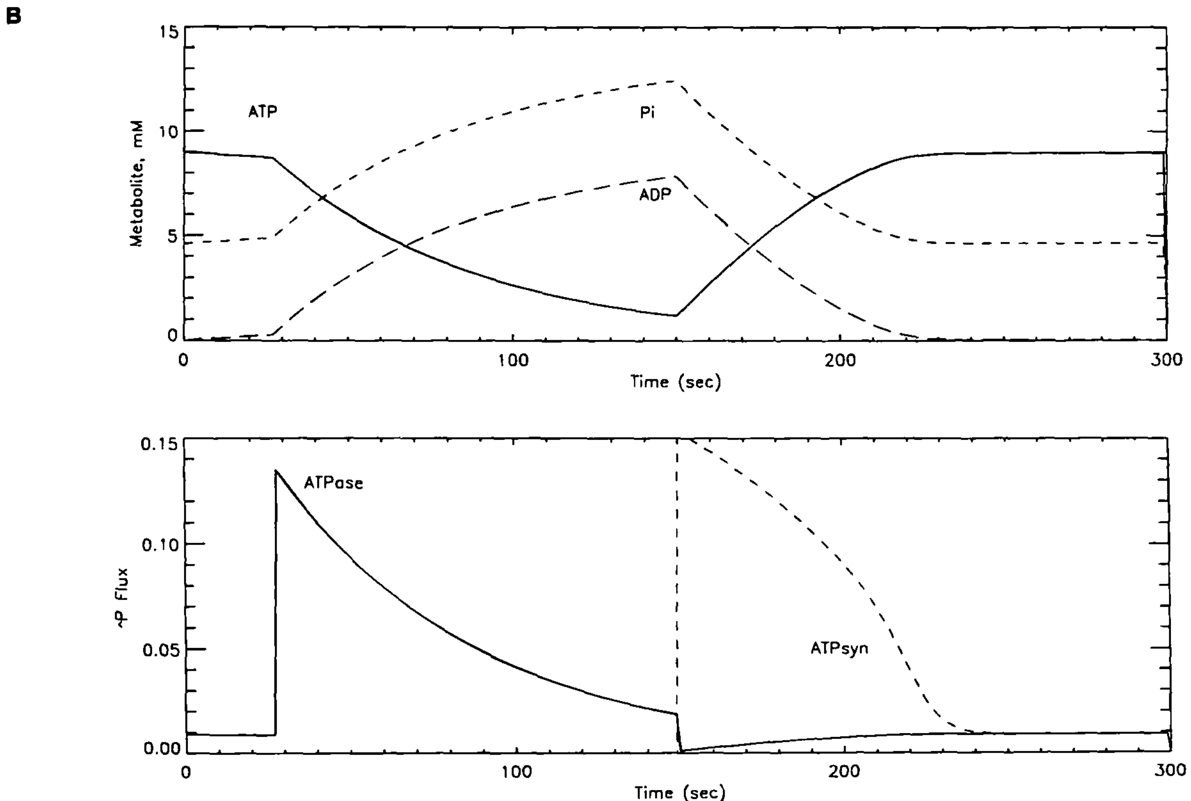

Fig. 4. Continued.

on the ATPase only. The model for oxidative phosphorylation uses simple Michaelis–Menton kinetics with substrates ADP and Pi. Both are cytoplasmic products of ATPases resulting in a "bare bones" feedback control model. Creatine kinase activity is determined solely by the concentration of its substrates and products; a full set of kinetic relationships were based on *in vitro* kinetic constants (Schimerlik and Cleland, 1973) as we reported (McFarland *et al.*, 1994). The simulations shown were made by numerical approximation of these relationships: for each time step, ATPase decreased [ATP] according to the first-order equation above. CK flux adjusted and set new [ATP], and [ADP] was calculated. ATP synthesis for that time step was calculated and the process was iterated for small time steps. Parameter values for metabolite levels, observed rates of PCr decline as a measure of the ATPase rate, and estimates of maximal flux of oxidative phosphorylation for our human forearm experiments were used (Blei *et al.*, 1993b).

First it is useful to simulate the case without creatine kinase; the results are displayed in Fig. 4. At 30 sec the ATPase rate constant was increased to increase ATPase in a step. [ATP] decreased with concomitant increases in [Pi] and [ADP]. The new increased flux of oxidative phosphorylation rapidly increased and reached a steady state in about 100 sec. The ATPase flux decreased with time because first-order kinetics was used and [ATP] decreased. At the time indicated, the ATPase rate constant was returned to the initial resting rate. The flux of oxidative phosphorylation returned to the initial (resting) condition within 40 sec for the model parameters used. For the conditions simulated, [ATP] decreased by about 2 mM with identical rises in [ADP] and [Pi]. This behavior is exactly that expected for the feedback model simulated, and is easy to understand. Panel B displays the results of a similar simulation in which oxidative phosphorylation was not operating during the interval of increased ATPase. This simulation was made because we will consider human muscle ischemic exercise below. It is clear that in the absence of any other source for ATP (CK function and the low rate of glycolysis was not included in the model), [ATP] plummets for the same $k_{contract}$ and duration in the simulation displayed in Panel B as compared to Panel A.

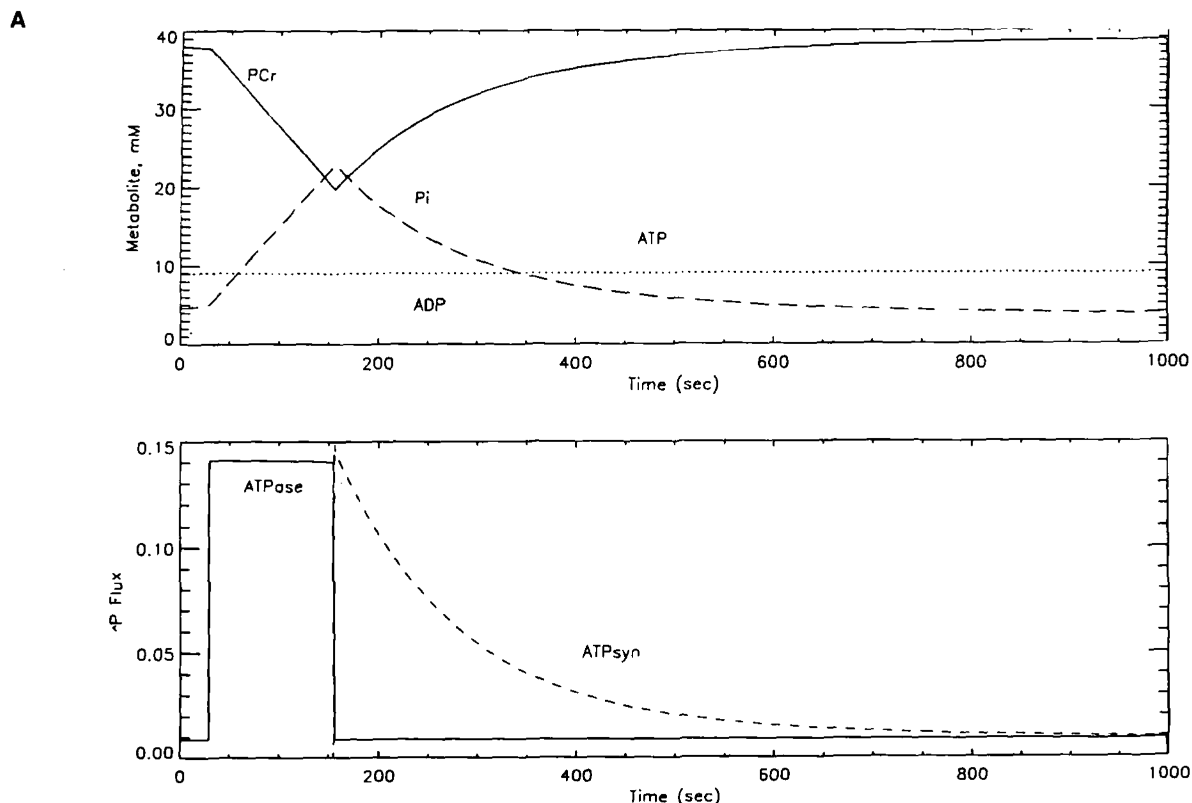

Fig. 5. The results of computer simulations based on the full model shown in Fig. 13.; these simulations include CK function in contrast to the results given in Fig. 4. Panel A displays the results obtained by simulating human forearm ischemic stimulation, similar to the experimental result shown in Fig. 1, but without an interval of continued ischemia after stimulation (high ATPase) ends. The linear decrease in PCr and increase in Pi without change in ATP is evident. There was a small increase in ADP to ~70 mM, but this is not seen on the scale used. The lower portion of Panel A shows the time course of the increased ATPase; the dashed line shows the time course of the rate of oxidative phosphorylation. Panel B displays the results obtained by simulating human forearm ischemic stimulation identical to the experimental result shown in Fig. 1. PCr continues to decline at the resting (low) ATPase rate during the 120 sec of continued ischemia before oxidative phosphorylation was begun at ~275 sec. The time scales differ between Figs. 1 and 5, but the close similarity of the simulation to the observed data is evident.

The magnitude of changes in adenine nucleotides and the rapid time course in this simulation is clearly different from simulations which include creatine kinase. Figure 5 displays the classical features of CK activity when CK activity was included in the simulations. The well-known buffering of changes in [ATP] and [ADP] is evident. The net observed reaction is the reversible splitting of PCr to produce Pi; on this scale shown, no changes in [ADP] or [ADP] are seen. Actually [ADP] increased to approximately 70 μM for the conditions simulated. It may be surprising to note that the kinetics of the responses of oxidative phosphorylation are slowed, but this is simply the result of a reduced magnitude of change in [ADP] per unit time for the kinetic function used. Thus, the addition of creatine kinase activity decreases the response time of the energy balance system. Other features of the CK system, such as distribution of isoforms, transport barriers in the mitochondria, and specific functions of Mi-CK have not yet been added quantitatively to this bioenergetic model scheme.

The complete functional role of CK isoforms in cells is still not fully defined as discussed in Section 2.2. Clearly the basis of our earlier analysis (Meyer *et al.*, 1984) and the present model could be expanded to consider the compartmentalized distribution of CK isoforms. To judge what the significance of adding these features might be, one must consider two cases: the mitochondrial function with and without any Mi-CK, and the mitochondrial isoform on the outer mitochondrial membrane (OMM). My conclusion will be that all exported ATP or PCr will come to local equilibrium with the local ADP, ADP, Cr, and PCr in the cytoplasmic spaces. We have argued that transport and

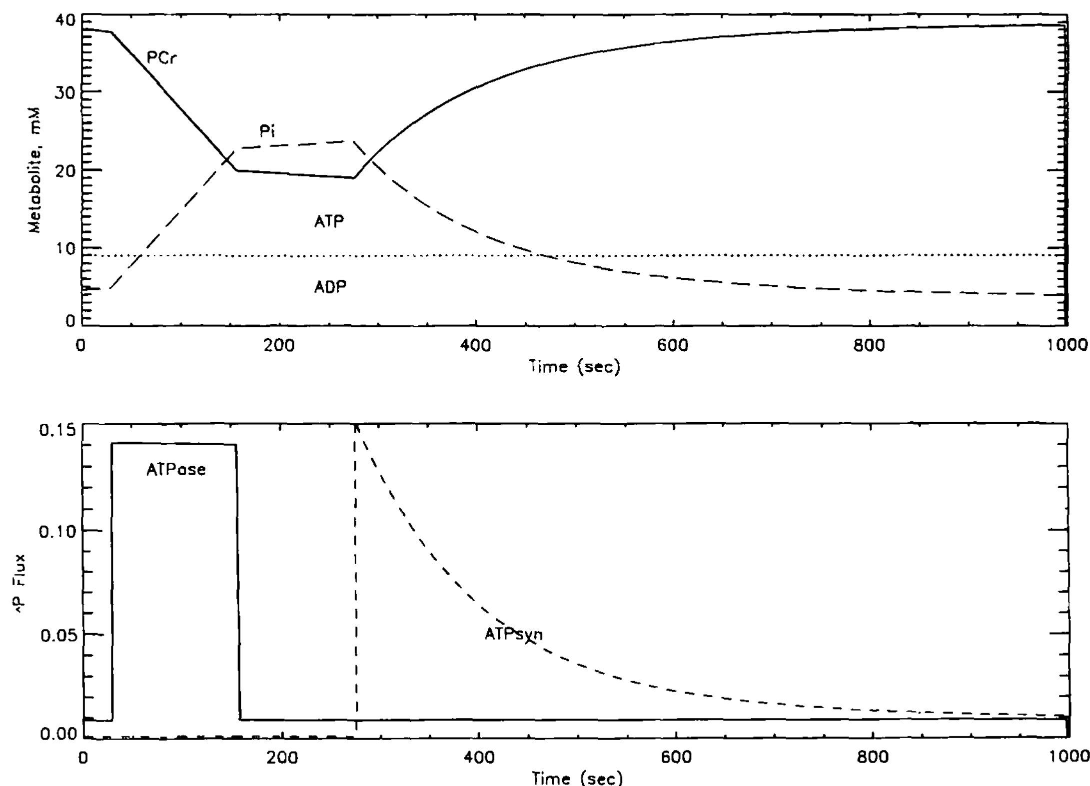

Fig. 5. Continued.

buffering are equivalent aspects of the same mechanism (Meyer *et al.*, 1984). Thus, the ratio PCr/Cr in the cytoplasmic space just outside of the mitochondrion (assuming those are the forms of transport in the presence of Mi-CK) or the local ratio ATP/ADP (if those are the chemical forms of transport by the adenine nucleotide transporter only) will be somewhat higher than the average throughout the rest of the cell, i.e., within several micrometers, given reasonable values for fluxes and diffusion coefficients. Consider mitochondria with Mi-CK and mechanisms for transport of only PCr and Cr across OMM. This PCr and Cr is in equilibration with the rest of the cellular ADP, ATP, Cr, and PCr in the cytoplasm but not necessarily at the same position of equilibrium in all mitochondrial compartments. Now consider mitochondria without Mi-CK; ATP and ADP are transported, and this flux generates the gradients just outside OMM as above. But this ATP and ADP will also equilibrate with the rest of the cellular ADP, ATP, Cr, and PCr as in the case of PCr and Cr transport. Thus neither transport mechanism leads to any differences in the cytoplasm which contains most of the metabolites and in which equilibration of the substrates and products of CK is known to occur. Of course, within the mitochondrion these models have significant differences, and it is these which may be responsible for differences in muscular performance in the experiments with transgenic mice in which the cytoplasmic CK isoform is deleted but Mi-CK is normal or increased in content.

Finally, we consider a simulation of our standard human forearm experiment. Panel B displays the result of simulations matching the conditions of our basic experimental observations, shown in Fig. 1. This calculation uses the identical model as in all the other simulations in Figs. 4 and 5. After stimulation and PCr decrease stopped, we continued ischemia for 2 min to test whether glycolytic recovery could be detected; it was not. This feature was simulated by inserting a period of basal ATPase rate between the contraction and the onset of oxidative phosphorylation. Then blood flow was restored and oxidative phosphorylation commenced. It is clear that the only difference between Panels A and B of Fig. 5 is that delay. It is also clear

that this simple model and simulation accounts for the essential features of the results given in Fig. 1. Occam's aphorism suggests that more complicated models are not needed, and likely will only fill in details. My main point is to emphasize that such a simple model reflects the main features of the observed results.

There are limitations of this sort of simulation of course, and it will certainly be useful to add known features and complexity, e.g., a more accurate mechanism for control of oxidative phosphorylation, a more realistic time course of contractile ATPase, inclusion of glycolysis and pH changes, and the functions of mitochondrial isoform of creatine kinase. Nonetheless, the successful implementation of the principles of bioenergetics in such a simple model does argue that the basic outline of control of energetics as developed in this essay is correct. The example drawn is from our recent experiments in human muscle. The use of the same model with appropriate values for metabolite concentrations, ATPase rates, and maximal rates of oxidative phosphorylation tests the generality of this mechanism in other systems.

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