Skeletal Muscle Energetics and Exercise Tolerance

Bioenergetics Group and The Physiological Society Joint Colloquium Organized by S. A. Ward (Centre for Exercise Science and Medicine, University of Glasgow), M. T. Wilson (Department of Biology and Chemical Sciences, University of Essex) and J. Wrigglesworth (Department of Clinical Biochemistry, King's College London), and Edited by S. A. Ward. 675th Meeting held at the University of York, 17–19 December 2002, Joint with The Physiological Society.

Energetics of muscle contraction: the whole is less than the sum of its parts

M. J. Kushmerick*† and K. E. Conley*†
*Department of Radiology, University of Washington, Seattle, WA 98195, U.S.A., †Department of Physiology and Biophysics, University of Washington, Seattle, WA 98195, U.S.A., and ‡Department of Bioengineering, University of Washington, Seattle, WA 98195, U.S.A.

Abstract
Understanding muscle energetics is a problem in optimizing supply of ATP to the demands of ATPases. The complexity of reactions and their fluxes to achieve this balance is greatly reduced by recognizing constraints imposed by the integration of common metabolites at fixed stoichiometry among modular units. ATPase is driven externally. Oxidative phosphorylation and glycolysis are the suppliers. We focus on their regulation which involves different controls, but reduces to two principles that enable facile experimental analysis of the supply and demand fluxes. The ratio of concentration of phosphocreatine (PCr) to ATP, not their individual values, sets the range of achievable concentrations of ADP in resting and active muscle (at fixed pH) in different cell types. This principle defines the fraction of available flux of oxidative phosphorylation utilized (at fixed enzyme activities). Then the kinetics of PCr recovery defines the kinetics of oxygen supply and substrate utilization. The second principle is the constancy of PCr and H+ (lactate) production by glycogenolysis due to the coupling of ATPase and glycolysis. This principle enables glycogenolytic flux to be measured from intracellular proton loads. Further simplification occurs because the magnitude of the interacting fluxes and metabolite concentrations are specified within narrow limits when both the resting and active fluxes are quantified. Thus there is a small set of rules for assessing and understanding the thermodynamics and kinetics of muscle energetics.

Introduction
Cells contain an enormous number of metabolic pathways that can give rise to a daunting complexity. Given this potential complexity, there are two approaches for discerning cell function. The first is to characterize the complexity by developing complete lists of component reactions and processes, and describing their interactions. A second approach is presented here: to uncover the underlying rules governing the function that this myriad of pathways must obey. Here we show that a minimal set of organizing rules greatly reduces the apparent complexity and accounts for a remarkable range of functions in resting and exercising muscle. Because these aspects of muscle energetics are so fundamental to the operation of all cells, we suggest that the rules presented here speak to the general problem of integrating molecular and cellular mechanisms into valid physiological systems.

We focus on two overriding biological principles of muscle energetics that lead to a number of underlying rules. The first principle is that mass...
The interconnections of the \( \text{O}_2 \), ATP and \( \text{H}^+ \) fluxes in actively contracting muscle are illustrated. We argue here that these principles permit us to obtain basic information concerning the organization of muscle energetics, and to discern simple, yet surprisingly powerful, rules governing function.

### Regulation results from integrated controllers

Achieving the balance of \( \text{O}_2 \) supply with mitochondrial demand and, in turn, of ATP supply with contractile demand requires control systems that sense need and activate supply. The control schemes for cellular events can be extraordinarily complicated, involving many intermediates, phosphorylation events, etc., but in muscle the entire process begins with the activation of ATPases in contraction. However, very simple schemes for controlling fluxes result from near-equilibrium reactions that act as simple integrators of supply with demand. These reactions form a feedback control system by generating a signal, activating supply, and integrating feedback between supply and demand. This feedback behaviour results from the near-equilibrium reaction acting as a buffer to maintain energetic or mass balance, which causes a shift in equilibrium that generates a signal that activates supply. Examples of near-equilibrium reactions that act as chemical buffers and also as integrators are the oxymyoglobin reaction, which keeps the cellular partial pressure of oxygen (\( \text{PO}_2 \)) buffered, and the creatine kinase (CK) reaction, in which the phosphocreatine concentration ([PCr]) varies with work rate to keep [ATP] constant. We illustrate these points in detail using the PCr/CK reaction, which is central to integrating oxidative ATP supply with ATP demand.

### Biochemical capacitors buffer metabolite levels

A biochemical capacitor is a generalization of an ordinary pH buffer reaction. Here we argue that the properties that permit chemical capacitance are also well suited for integrating cellular fluxes. Such a reaction is poised at equilibrium with regard to its reactants and products, so that addition or removal of the moiety being buffered is compensated by adjustments in the other components in the buffer reaction. The CK reaction in the cytoplasm is a good example of this buffering, as cellular ATP levels are maintained constant in the face of changing demand because of buffering by PCr:

\[
\text{PCr} + \text{ADP} + \text{H}^+ \leftrightarrow \text{creatine} + \text{ATP}
\]  

(1)

Increased ATP demand due to muscle contraction is met by a shift in the CK reaction that decreases [PCr] without a change in [ATP]. This equilibrium shift keeps [ATP] (and the free energy of ATP) relatively constant over a wide range of ATP fluxes in the cell. Thus PCr is the chemical capacitor for ATP, and the CK reaction has several properties in common with all buffer reactions. (1) The flux capacity of the buffer reaction must be greater than the flux of the reaction being buffered.
The activity of CK has been measured in living skeletal muscle cells by direct 31P NMR spin transfer methods, and its flux is much higher than either the maximal ATPase or the ATP synthesis flux [1]. (2) The buffer reaction must be at, or close to, equilibrium with regard to its reactants and products. The capacity of all buffers is greatest when poised near their equilibrium. Otherwise the reaction will not be a buffer, but will drive the reaction in one direction or the other. (3) The concentrations of PCr and creatine reflect the position of equilibrium. This is defined by the equilibrium constant and the concentrations of the other reactants and products. Thus a measure of the cellular [PCr]/[creatine] ratio at a known pH gives a good measure of the [ATP]/[ADP] ratio. [PCr] is a sensitive indicator of the energetic status of the cell, because changes in ATP flux cause a shift in the CK equilibrium that is reflected in the [PCr].

**Buffers as metabolic integrators**

The CK reaction integrates ATP demand and oxidative ATP supply. The double role of the shift in [PCr] in buffering short-term ATP demands and elevating the signal for longer-term oxidative ATP supply illustrates the integrative role of the CK reaction. Thus a key feature of the buffer action of CK central to muscle energetics is that it links PCr dynamics to mitochondrial function.

Another near-equilibrium reaction involved in integrating flux information for metabolic regulation is the adenylate kinase reaction, which links AMP formation to ADP generation. As ADP levels rise, [AMP] also rises to levels that activate two metabolic pathways: glycolysis and glucose uptake [3]. This activation of substrate uptake and catabolism is particularly important for hypoxic resting muscle that requires a glycolytic ATP supply.

An independent, near-equilibrium reaction involving buffering and flux regulation is the oxygen/myoglobin reaction. Myoglobin is found at high concentrations in muscle and has a high but reversible affinity for oxygen. This high affinity and the myoglobin concentration in muscle means that most of the O2 in the muscle cell is bound to myoglobin. Similar to the role of PCr in keeping [ATP] constant, intracellular Po2 is buffered by oxymyoglobin, with any change in Po2 countered by O2 release from oxymyoglobin. Finally, Po2 appears to be an important regulator of O2 delivery. Thus a drop in intracellular Po2, indicating a net loss of O2, signals an increase in blood flow and enhanced O2 delivery. These examples illustrate how simple, near-equilibrium reactions can act as integrators of the supply and demand of energy or mass in cells.

**Metabolite ratios normalize regulatory signals**

Simple integrators illustrate how to link energy demands to energy supply in a quantitative manner. How can integrators work when metabolites that generate the signals, such as PCr, vary substantially in total content among different muscles and among individuals? Atkinson [4,5] provided insight into this problem when he defined the ratio of ATP, ADP and AMP concentrations as the 'adenylate charge' governing metabolic regulation. The CK reaction accomplishes this scaling in muscle (and for any cell containing CK or arginine kinase) for the regulator of oxidative phosphorylation. We showed that the [PCr]/[ATP] ratio is approximately constant,
Despite wide variations in [PCr] and [ATP] in different types of mammalian muscle cells \([6,7]\), the non-linearity of respiratory control by ADP is the best known and most widespread example of a common intermediate whose role is to carry chemical energy. Cell function depends on adequate supplies of ATP, because ATP is the form of chemical potential energy that is used to drive every kind of cellular work. This use of ATP by the cells must be balanced by an equal synthesis, which leads to the notion that cellular energetics are best analysed as a supply and demand problem.

The constraint on the muscle energy system is that demand cannot outstrip supply. The turnover of the ATP pool during exercise may occur many times per minute; this requires that ATP usage be balanced by ATP synthesis from moment to moment. In steady-state contractile activity, under which condition one can demonstrate steady concentrations of PCr, Pi, ATP, ADP and pH, the rule of mass and energy balance requires that ATP synthesis matches demand. Measuring one component gives the other. The interconnections among the major metabolic components are illustrated in Figure 1. The basis of these links is the stoichiometric relationships in the chemical reactions and among groups of reactions in pathways, as described below.

**Rules based on stoichiometry of metabolic reactions**

The basis for the central role that ATP plays in energy metabolism and for the links and balances described above (Figure 1) resides in reaction stoichiometry. Reaction stoichiometry is defined by the chemical nature of the reactions. These result from the laws of chemical combination and mass conservation, which have been well analysed by stoichiometric matrices \([9]\). Reaction stoichiometry forms the mechanistic basis for the links shown in Figure 1. In a set of reactions that function together, such as the combined operation of ATPase and CK, there is a fixed stoichiometry between the reactants and products, including \(H^+\). The stoichiometric coefficients depend on pH and on cation binding, as is well defined from known physical chemistry \([10,11]\). Provided that major cations in muscle cytoplasm do not change their concentrations, which is the normal physiological condition, these stoichiometric coefficients are definable functions of pH. The functional significance is the practical use one can make of the relationships among changes in phosphate-containing compounds, their fluxes, and \(H^+\) load and intracellular pH. Application of stoichiometric rules works for entire pathways. For ex-

---

**Mass and energy fluxes must balance**

The fact that mass and energy must be conserved provides useful, simplifying rules. The connections of the major mass and energy fluxes in the cell, illustrated in Figure 1, provide one class of simplifications that are useful in understanding metabolism.

**Link between \(O_2\), ATP and \(H^+\) balances**

The links between these pathways take several forms. The first form, evident in Figure 1, is the serial nature of the three fluxes. The demand for ATP by the muscle sets the demand for \(O_2\), which in turn sets the demand for blood flow. Thus knowledge of one flux specifies the size of all other linearly connected fluxes in steady state. An additional feature of the connection between fluxes is that the pathways share intermediates. Lipmann \([8]\) realized that, of the thousands of possible substrates and products, only a small number are common intermediates in multiple pathways, and only some of those are involved in the storage of chemical potential energy. ATP
ample, in any cell the complete oxidation of glucose requires six molar equivalents of O\textsubscript{2}. Another example is glycogenolysis and glycolysis. The proton stoichiometric coefficients for glycogenolysis and glycolysis coupled with the CK reaction and that for glycolysis from glucose are defined in [11]. We and others have taken advantage of these stoichiometric relationships to work out the extent and rate of glycogenolysis and glycolysis in muscle contraction [12–15]. These rules allow extraction of information on these fluxes directly from \textsuperscript{31}P NMR spectroscopic data.

There are other stoichiometric relationships established empirically; that is, there are sets of reactions that include other factors in addition to fixed reaction stoichiometry. The most relevant of these for muscle energetics is oxidative phosphorylation. The P/O\textsubscript{2} ratio has been measured in frog and mammalian skeletal muscle, and found to be constant over a range of contraction conditions [16]; a similar ratio is observed in renal tissue ([17], but see [18–20] for other results). The P/O\textsubscript{2} ratio of ~6 is well predicted by the oxidation of carbohydrate and substrate level phosphorylation without considering leaks and uncoupling. The important aspect of these results in active muscle is that the stoichiometry appears to be constant, independent of the physiological state, over the normal range of function. Application of stoichiometric rules allows the components, as shown in Figure 1, to be related to each other quantitatively. In this way, ATPase flux can be shown to predict O\textsubscript{2} flux. Stoichiometric rules provide simplifying guidelines to understanding all of these relationships.

Summarizing remarks

We argue here that the principles described provide basic information concerning the organization of muscle biochemistry, and greatly simplify our understanding of the integrated energetics of muscle function. The first insight is that near-equilibrium reactions offer a simple mechanism of flux regulation. The candidates and possibilities needed for a mechanistic understanding of oxidative phosphorylation are narrowed. Control of cellular respiration by ADP, as indicated by the status of the CK reaction, is one example where the simple approach has clearly identified the players, the system and their interactions in the control of cell respiration. Full control includes other mechanisms, but the simple integrator represented by these reactions provides considerable insight into the control scheme. The second insight is that fluxes are closely linked and constrain whole-system behaviour. Knowledge of the contractile ATP demand sets the limit on supply of ATP, O\textsubscript{2} and blood flow, because muscle contraction is a demand–regulated system. Thus we can start modelling and making mechanistically realistic and quantitative predictions about intracellular energetics in a parsimonious manner. The output of relatively simple models make intuitive sense, can be tested against experimental data, and can be used to make realistic and quantitative predictions about intracellular energetics in novel situations.

References


Received 23 November 2001