A PARADIGM FOR ENERGY BALANCE IN MUSCLE FUNCTION

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INTRODUCTION

This essay considers energy balance in muscle: what it means and why it must be achieved in normal muscle functions. The essence of the problem can be simply stated. Work is done by the muscle only by using ATP. Oxidation of metabolites is necessary to do the work of ATP synthesis. The amount of ATP utilization and ATP synthesis exactly match - a statement of energy balance akin to conservation of energy but stated in biological observables.

MYOTHERMAL AND BIOCHEMICAL ENERGY BALANCE

The classical approach to describe and understand energy balance was defined by AV Hill [1,2] by the application of energy conservation in physical terms to contracting muscle. The conceptual basis of myothermal energy balance states that the total energy change between two states of a muscle is exactly equal to the sum of the heat output and the work done in that change. To apply this first law of thermodynamics to muscle one must identify all the metabolic reactions, know their molar enthalpies and quantify their extents of its change, and of course measure the heat produced and the work done in defined states [3]. The many successes of this approach [4,5] have been displaced by elaboration of so many individual metabolic reactions, including highly exothermic and endothermic ones, that the task of quantifying their extents of reaction and enthalpies led to unmanageable experimental difficulties and uncertain correlations of the details of the chemical processes with myothermal events. The burden in analyzing energy balance from a biochemical energy balance view [6] is to work out the principles that organize the operation of the metabolic pathways and govern their functional integration in maintaining a chemically defined energy balance (as distinct from myothermal energy balance defined physically). Identification of the metabolic processes and measuring their extents of reaction in muscle activity is required in any event and uses specific and sensitive enzymatic assays and more recently physical methods (nuclear magnetic resonance and optical fluorescence and spectroscopy).

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 [7]. 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. Energy balance concerns the function of that anatomy. Energy transductions in muscle are carried out by molecular electro-chemical and chemo-osmotic transducing machines and chemo-mechanical motors. These synthesize or dissipate ATP, the cell's source of chemical potential energy, in a way that couples the energy to the metabolic, electrical, osmotic and mechanical work. The extent of energy dissipation uncoupled to ATP is negligible. Although a small amount of ATP is stored in the cell (on the order of 5 mM), ATP must be produced

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by metabolism concurrent with work performance. The reason is that typical rates of ATP utilization in active muscle (on the order of 1 $mM \cdot s^{-1}$) would otherwise exhaust the muscle's source of chemical potential energy in tens of seconds, at the longest. We know that steady states of metabolic activity are possible over a wide range of muscle functions from a basal minimum up to some maximum, beyond which the integrated operation disintegrates into dysfunction for which we use terms like fatigue, pathology, disease, etc. This range of more than ten-fold in coupled metabolic power requires for our understanding a set of principles which organize the facts and govern their regulation. Lists of enzymes, their regulatory mechanisms and associated sets of differential equations have failed to accomplish this task. I believe the reason is the lack of organizing principles and analytical structure. Energy balance describes those properties of energy using and generating mechanisms and their control that sets the supply of chemical energy to match the demands of chemical transducing machines.

My goal is to define a set of principles equivalent to those of thermodynamics of heat engines. These are not *a priori* predictable from first principles of physics because the mechanisms involved are the product of evolution, but presumably they can be defined, understood and integrated into a quantitative synthesis and comprehensive model. The task is trans-disciplinary in the sense that it moves beyond the integration of various disciplinary boundaries to achieve a new set of operational principles. The following statements proceed from facts and nearly self-evident principles to working and spectulative hypotheses, and these comprise a beginning in the quest for this set of principles.

A SET OF OPERATIONAL PRINCIPLES

1. ATP provides the energy for all forms of muscle work

There is no other form of energy coupling known; muscle is not a heat engine or fuel cell. The essential feature of muscle as a chemomechanical system is that Gibbs energy of chemical reactions is coupled to mechanical performance by the actomyosin motor. Chemical input power matches the mechanical output power. Actomyosin interactions generating force and doing work (shortening against a load) 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. Some important questions follow from this statement. Does the power of the actomyosin molecular motor depend on the magnitude of the chemical potential? In isometric conditions, the force produced per unit ATP utilized appears to be independent of the concentrations of ATP, PCr, Cr, P_i and pH. That is, there was a constant rate of ATP utilization proportional to the integral of isometric force independent of the changing content of high energy phosphate compounds over a range in which most of the PCr was depleted in amphibian [8] and mammalian muscle [9]. That is, the cost per unit mechanical output was constant although the chemical potential decreased. Experiments answering similar questions about the quantities of work done per unit ATP utilization (see *Fig. 4.37* of [5]) and the comparison of chemical power input per unit of mechanical power output for working muscle [10] indicate a matching of ATP utilization to mechanical work output, also in constant proportion although the chemical potential decreased over the range of measurements. Thus it appears that the linear flow-force relationships of some non-equilibrium thermodynamic formulations are not observed.

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. One example is nucleotide diphosphokinase which catalyses the exchange between ATP and other purine and pyrimidine nucleotide triphosphates:

$$ATP + XDP \leftrightarrow XTP + ADP \qquad (1)$$

Another is the coupling of redox potential (NAD^+/NADH) with phosphorylation potential by glyceraldehyde-phosphate dehydrogenase, and other metabolic sites. Perhaps the best known example is creatine kinase [11,12] which catalyses the interconversion between ATP and phosphorylcreatine (PCr):

$$ATP + Creatine \leftrightarrow PCr + ADP + H^+ \qquad (2)$$

This reaction introduces the principle of a chemical energy capacitance. The content of PCr is effectively a capacitor of chemical energy in combination with creatine kinase, by the nature of its near equilibration with its substrates and products. Muscle cells, and excitable cells in general, have significant concentrations of PCr often higher than of ATP. 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. The important point is that, as a capacitor, PCr represents chemical potential energy previously synthesized by oxidative metabolism. Thus endergonic processes can be temporarily coupled to Gibbs energy dissipation without the requirement for simultaneous oxidative metabolism provided the limits of the chemical energy capacitance, the supply of PCr, is not exceeded. The definition of the system for energy balance must be made carefully. If the system is considered to span ATPase to oxidative metabolism, then the dissipation of the energy in the capacitor can be an imbalance in terms of oxygen consumption, as the term 'oxygen debt' infers. A definition of the system as ATPase and CK has no energy imablance. Of course less Gibbs energy is dissipated the closer the CK reaction is to equilibrium and the concept of chemical capacitance depends on near equilibrium. However it is not known experimentally just how close to equilibrium the CK reaction remains during rapid transitions from rest to work.

3. The sum of the coupled ATPases sets the demand side of the balance and defines energetic states

The rate of the coupled Gibbs energy dissipation mechanisms is the primary and causal mechanism in bioenergetics. The overall metabolic rate of a cell changes because its ATPase rates change; the converse is also true, *viz.* that if a measure of the overall metabolic rate increases then that means the steady state sum of the ATPases have increased. The ATP dissipating mechanisms (coupled to performing work) are in turn activated by signals external to this molecular motor. In this view 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. Skeletal muscle is 'the servant of the nervous system', meaning that activation of the ATP utilizing machines originates by physiological

mechanisms and events from outside the muscle cell. Once this cascade of events occurs in striated muscle, ATP hydrolysis is coupled to mechanical work. Otherwise the ATPase rate is extremely low. The magnitude of ATP hyrolysis uncoupled to the molecular transducers (in analogy to the proton leak dissipating chemical energy in the mitochondria without coupling to ATP synthesis [13]) is unknown, and, on the basis of the 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, and other energy-using processes. The total energy required for all of these processes is then equal to the sum of ATP utilization. This energy demand is one side of the equation of energy balance. Unanswered experimental questions are whether all of these processes are independent or dependent on the chemical potential of ATP at the time of energy transduction. We concluded above that the actomyosin motor behaves independently.

4. The coupled 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. How is this constancy achieved in the face of varied levels of demand? One possible way is that the signals which activate the ATP utilizing mechanisms also provide information to the coupled processes synthesizing ATP. These different molecular processes activated by a common external signal could result in energy balance by a common signal acting on the ATP utilizing and synthesizing mechanisms in parallel. This mechanism is essentially the role of calcium activation of mitochondrial dehydrogenases [14,15]. Despite the existence of such feedforward mechanisms, it is causally and mechanistically necessary that a signal (or signals) derived from the coupled chemomechanical machine acts as a feedback regulator of metabolic ATP synthesis. The reason is a biological and logical necessity. Since energy balance is a cellular process, it is a better strategy, teleologically, to have the regulation self contained. For this to occur, the primary signal molecules must be one or more of the chemical products of ATP-coupled molecular machines: inorganic phosphate (P_i), ADP, H^+ or creatine. These internal signals in turn regulate intracellular ATP synthesis by feedback signals, whether kinetic or thermodynamic control of oxidative phosphorylation is considered. The presence of feedback control does not negate the existence of feedforward ones. The feedback mechanisms derived from the coupled ATP dissipating mechanisms are essential to achieve energy balance if the result of the feedforward signals were in the slightest way imbalanced. Without operating feedback control such imbalances could not be restored. Tests of pure feedback control predict that measured quantitative relationships (kinetic formulations of ATP synthesis velocity versus ADP concentration, or themodynamic ones of ATP synthesis or mechanical power output versus ATP chemical potential) are independent of the intracellular physiological conditions. On the other hand, feedforward mechanisms involve alterations of enzyme activities or substrate affinities, such that apparent kinetic constants, maximal velocities, or coefficients of thermodynamic relationships would be dependent on intracellular physiological conditions activating such signals. Such experimental designs and results are urgently needed.

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

Energy balance must be satisfied even if a muscle transiently depends on its 'high energy phosphate' capacitor PCr, instead of new ATP synthesis. This imbalance can happen when the magnitude of the ATPases exceeds the maximum of the ATP synthesis. I now consider examples in which the preceding sections can be shown to have explanatory and predictive power. It is well known that muscles differ in their kinetics, and in their mechanical and metabolic power. 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 muscle faster mechanically has a greater rate of coupled ATPase than a slower muscle. Similarly muscles differ in their mitochondrial content such that their maximal rate of substrate oxidation and oxidative phosphorylation differ. We have come to recognize these differences as stereotypes, *e.g.* fast twitch glycolytic or slow oxidative muscle types in what is likely to be a continuum of properties [16,17]. The range of these rates in mammalian muscle is on the order of 2- to 4-fold for each side of energy balance. If the cell uses all of its stored energy its function fails - we commonly refer to this state as fatigue wherein some or all of the same feedback signals are inhibitory for ATP utilizing mechanisms. ATP synthesis balancing ATP use may then be separable in time. A well known example of this case is a maximally activated fast twitch muscle, but no steady state is then possible. Consider the opposite case in which the size of the ATP synthesis components greatly exceeds the magnitude of the ATPases. Then muscle activity will appear as if there is little or no feedback error signal operating for two reasons (besides the idea that the regulation and control in different types of striated muscle, cardiac and skeletal, differ qualitatively - *e.g.* [18]). First the size of the error signal in this example will be small and perhaps within the noise of our experimental measures. Secondly the effective 'gain' in the system is large so that small signals produce big effects. A well known example of the second case is cardiac muscle. One value of the view proposed here is that a common regulatory scheme can be considered in general, with differences between muscle types being quantitative, not qualitative. Note finally that for the same amount of energy utilization (*e.g.* mechanical power), energy balance is obtained with different biochemical states: larger change in 'high energy phosphates' in the case of mitochondrial poor muscle and smaller change in the case of muscles rich in oxidative metabolic machinery.

6. Mechanisms of energy balance control muscle plasticity

The phenotype of adult muscle is highly adaptable to stresses and depends on historical pattern and intensity of usage [19]. An argument from parsimony extends the control of ATP synthesis to mechanisms of gene expression. The observation that slow twitch glycolytic muscle stereotype has not been observed in Nature suggests there may be an interaction between quantitative energy balance in bioenergetics and muscle phenotype. Feedforward control of ATP synthesis relegates the control of muscle phenotype to mechanisms outside of the muscle; many of these external influences are known (*e.g.* thyroid hormone) and are certainly important during development and in the adult. However the major effect in normal physiological conditions is that of persistent muscle activity on isoform

expression in the molecular transducing machines. This makes control of gene expression by feedback signals originating from the components of energy balance an attractive possibility to consider. Hypoxic states can be strong stimuli to altered gene expression [20,21]. Alterations of the cellular 'high energy phosphate' content by creatine analogs is associated with altered expression of myosin heavy chains [22], metabolic enzyme profiles [23] and mechanical function [24]. These several observations suggest a speculative scheme in which altered bioenergetic states play a mechanstic role in the complex processes of maintaining and altering the appropriate protein expression to achieve the various phenotypes known in adult muscle.

CONCLUSION

This set of six statements and working hypotheses provides a relatively simple scheme by which one can integrate the diverse component mechanisms of bioenergetic systems such as muscle cells. These are tentative rules and hypotheses which bring novel approaches to experimental questions, and are extensible to higher order hierarchical systems such as the interplay between liver and muscle in exercise with respect to carbohydrate and lipid metabolism.

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