

## CELLULAR METABOLIC HOMEOSTASIS DURING LARGE-SCALE CHANGE IN ATP TURNOVER RATES IN MUSCLES

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### Summary

The term homeostasis traditionally refers to the maintenance of a relatively constant internal milieu in the face of changing environmental conditions or changing physiological function. Tissues such as skeletal and cardiac muscles must sustain very large-scale changes in ATP turnover rate during equally large changes in work. In many skeletal muscles, these changes can exceed 100-fold. In unique biological circumstances (for example, during periods of oxygen limitation, vasoconstriction and hypometabolism), tissues such as skeletal muscles may be obliged to sustain further decreases in ATP turnover rates and operate for varying periods at seriously suppressed ATP turnover rates. Examination of a number of cellular and whole-organism systems identifies ATP concentration as a key parameter of the interior milieu that is nearly

universally 'homeostatic'; it is common to observe no change in ATP concentration even while the change in its turnover rate can increase or decrease by two orders of magnitude. A large number of other intermediates of cellular metabolism are also regulated within narrow concentration ranges, but none seemingly as precisely as is [ATP]. In fact, the only other metabolite in aerobic energy metabolism that is seemingly as 'homeostatic' is oxygen – at least in working muscles. The central regulatory question is how such homeostasis of key intermediates in pathways of energy supply and energy demand is achieved.

Key words: homeostasis, muscle, ATP turnover, metabolism, regulation.

### Introduction

#### *Contrasting demands of homeostasis and muscle work*

As traditionally defined, the term homeostasis refers to the constancy of the internal milieu in the face of external perturbations; the latter, in principle, may be caused by extracellular factors or, in the case we shall consider, by changes in intracellular biological function (Hochachka and Somero, 1984). Of all tissues in the vertebrate body, skeletal muscle displays the special quality of being able routinely to sustain very large changes in work and metabolic rates. Compared with the 1.5- to twofold differences in metabolic rates between resting metabolic rates (RMRs) and activated states, which are common to many tissues (liver and brain, to mention two), skeletal muscles in humans must be able to sustain up to, or even over, 100-fold changes in ATP turnover rates. Amongst vertebrate endotherms, the highest muscle metabolic rate (in the range of  $600 \mu\text{mol ATP g}^{-1} \text{min}^{-1}$ ) appears to be that of hummingbird breast muscle during hovering flight – a rate over 500 times muscle RMR (Suarez, 1992; Suarez *et al.* 1990, 1991). During muscle ischemia, hypoxemia or hypoxia, the muscle, like many other tissues under conditions of oxygen lack, may need to sustain a suppression of metabolism even below resting rates (Hochachka and Guppy, 1987), thus even further extending the enormous range between the lowest and highest sustainable ATP turnover rates of this remarkable tissue.

Current popular interpretations of how these kinds of large-scale differences in steady-state energy turnover are regulated assume cybernetic feedback control circuitry. The standard theory is summarized in Fig. 1 (see Balaban, 1990; Chance *et al.* 1986; Connett, 1988; Connett *et al.* 1985; Connet and Honig, 1989; From *et al.* 1990; Funk *et al.* 1990; Kushermick *et al.* 1992; Rumsey *et al.* 1990). Following activation signals arriving at the muscle cell, an increase in ATP demand 'turns on' cell ATPases whose catalytic function leads to increased product (ADP,  $P_i$ ,  $H^+$ ) concentrations; the latter then serve as substrates and as positive feedback signals for accelerating ATP supply pathways (Fig. 1). Metabolites such as ADP and inorganic phosphate ( $P_i$ ) are thought to be pivotal in mitochondrial metabolic control, but powerful activation of cell work also demands a proportional activation of catalytic function at essentially every enzyme step involved in the ATP-supply and ATP-demand pathways. Hence, if substrate, product and modulator concentration changes are to be the main mediators of large (100-fold or more) changes in ATP turnover rate, one would anticipate equally large perturbations in pool sizes of numerous intermediates. This would be especially true for regulation processes based on Michaelis–Menten kinetics, where the kinetic order cannot exceed 1 (Atkinson, 1977, 1990); i.e. percentage change in catalytic rate (ATP turnover rate) cannot exceed percentage

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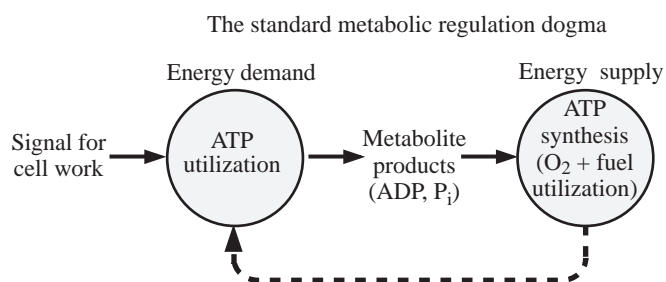


Fig. 1. Schematic diagram of the most common model for integrating ATP demand and ATP supply pathways, on the basis of simple feedback control loops. See text for details.

change in substrate concentration driving the metabolic change (Hochachka and Matheson, 1992; Hochachka, 1994). Whereas 'homeostasis' demands 'constancy of the internal milieu', muscle work would appear to require drastic changes in intracellular conditions, the degree of perturbation being somehow related to the intensity of work. The problem (and paradox) we wish to consider is how the conflicting demands of homeostasis *versus* metabolic regulation are resolved in muscle during different work and metabolic states; i.e. how muscles sustain *both* metabolic homeostasis and metabolic regulation. A good place to start our analysis is with a reappraisal of the mechanisms of metabolic regulation in human muscle.

#### Regulation of human muscle metabolism during work

A recent study (P. S. Allen, G. O. Matheson, G. Zhu, D. Ghergiu, R. S. Dunlop, T. F. Falconer, C. Stanley and P. W. Hochachka, in preparation) using noninvasive magnetic resonance spectroscopy (MRS) technology can be used to illustrate the situation. In this research, muscle  $[H^+]$  was calculated from the exchange-averaged or time-averaged chemical shift difference between monoprotonated and diprotonated phosphate. The concentrations of free ADP ( $[ADP]$ ) were calculated from the equilibrium constant for creatine phosphokinase (CPK), on the basis of recent studies assumed to be  $1.77 \times 10^9$ , with free  $[Mg^{2+}]$  taken to be  $1 \text{ mmol l}^{-1}$  and unchanging with exercise; this calculation takes into account the effects of pH on the calculated  $[ADP]$ . Also, for these calculations, we assumed a resting  $[ATP]$  of  $6 \text{ mmol l}^{-1}$  and a total creatine pool ( $[PCr] + [Cr]$ , where Cr is creatine and PCr is phosphocreatine) of  $24 \text{ mmol l}^{-1}$ , 75% phosphorylated (values well within the range expected for muscles of humans and other mammals). Any error in these estimates would change the calculated value of  $[ADP]$  but would not alter the fractional changes in concentrations during rest–work–recovery transitions, and it is the latter information which is most relevant to this analysis. To minimize between-subject variations, relative PCr concentrations were calculated as  $\{PCr\}$ , defined as the area under the PCr peak divided by the combined areas under the PCr,  $\beta$ -phosphate of ATP and  $P_i$  peaks. ATP turnover rates during different metabolic states could not be determined directly in these studies; hence, the ATP turnover rates were treated as a percentage of the maximum sustainable rate, analogous to

percentage of maximum voluntary exercise. However, it is known (i) that the ATP turnover rate during sustained submaximal muscle exercise is a direct function of the work rate (Arthur *et al.* 1992), which is why the latter can be used as an index of the former (Nioka *et al.* 1991), and (ii) that during work protocols involving small muscle masses in man (Saltin, 1985; Andersen and Saltin, 1985), the maximum ATP turnover rate is high (because cardiac output can be preferentially directed to a small working area). Assuming a similar maximum rate of about  $100 \mu\text{mol g}^{-1} \text{ min}^{-1}$  for these studies indicates muscle exercise intensities equivalent to ATP turnover rates of 20, 30 and  $40 \mu\text{mol g}^{-1} \text{ min}^{-1}$  in each of the three work episodes examined; these are approximately 20, 30 and 40 times muscle RMRs (approximately  $0.5\text{--}1.5 \mu\text{mol g}^{-1} \text{ min}^{-1}$  for both slow-twitch and fast-twitch muscles).

The data on PCr and  $P_i$  for the gastrocnemius were similar to data previously found (Matheson *et al.* 1991) for exercising muscles: declining  $\{PCr\}$  during exercise with a concomitant rise in  $\{P_i\}$ , followed by rapid recoveries during each subsequent rest interval. The chemical shift for  $P_i$  also showed a modest adjustment, indicating a modest change in the equilibrium between diprotonated and monoprotonated phosphate. The three ATP peaks, in contrast, remained stable throughout the protocol. For soleus muscle, all metabolite concentrations seemed to be more stable during exercise than those in gastrocnemius.

We analyzed the data using ATP turnover rate (assumed to be proportional to muscle exercise intensity) as the independent parameter. In the case of the gastrocnemius (Fig. 2), the change in ATP demand or in the work rate is linearly reflected in declining PCr concentrations. Since change in  $\{P_i\}$  is essentially stoichiometric with change in  $\{PCr\}$ , a good relationship is also observed between  $\{P_i\}$  and ATP turnover rate. However, it will be clear from Fig. 2 that the relationship extends far beyond the apparent  $K_m$  for  $P_i$  of mitochondrial metabolism (see Arthur *et al.* 1992); as with the  $\{PCr\}$  data, a kinetic order of 1 is not observed (Fig. 2). For these reasons, it appears that both  $\{PCr\}$  and  $\{P_i\}$  reliably reflect the ATP turnover rate demanded by the imposed exercise, but do not regulate the ATP turnover rate through effects on mitochondrial metabolism.

In contrast to the simple linear relationships between PCr and  $P_i$  concentrations and ATP turnover rates, the relationships between  $[ADP]$  and gastrocnemius work intensities are complex (Fig. 2C). Although the changes in ADP concentration are consistent with some role in metabolic activation (Kushmerick *et al.* 1992), the increase in  $[ADP]$  is not a simple and direct (1:1) function of work rate, as would be required by the Michaelis–Menten models of ADP control that are popular in this field. This difficulty with ADP as a primary regulator of ATP turnover rate is also noted elsewhere (see Balaban, 1990; Hochachka *et al.* 1991, 1996; Arthur *et al.* 1992; Hochachka, 1994). Such rest–work comparisons mean that the fractional changes in ADP concentrations seem to be much smaller than the fractional changes in ATP turnover rates in gastrocnemius muscle. It is unlikely therefore that the former could 'drive' the latter; again, it may be more realistic to view changes in  $[ADP]$

as reflecting changes in ATP demand by muscle ATPases (Hochachka *et al.* 1991; Hochachka and Matheson, 1992). Be that as it may, the data clearly emphasize that in the case of the gastrocnemius no simple causal relationships exist between imposed exercise (ATP turnover rates) and ADP concentration changes, and the same applies to {PCr} and {P<sub>i</sub>}.

For the soleus, the case for regulation of ATP turnover rate by any of these metabolites is even weaker than for the gastrocnemius. The kinetic order is further from 1 in the case of PCr and P<sub>i</sub> – even if both PCr and P<sub>i</sub> consistently reflect the differing exercise intensities (Fig. 2). With regard to [ADP], the soleus sustains the three exercise intensities at essentially constant ADP concentrations.

Taken together, we interpret these results to mean that, in muscles formed mainly of fast-twitch fibers, ADP and P<sub>i</sub> may play a fine-tuning role in regulating ATP turnover rates, but that some other (currently unknown) coarse-control mechanisms must be operative in controlling large-scale changes in ATP turnover rates during muscle work (also see Hochachka, 1994). In contrast, in slow-twitch oxidative fibers (which dominate soleus muscle), neither ADP nor P<sub>i</sub> seems to be of any particular regulatory significance – a situation rather reminiscent of that in the heart of large mammals (Balaban, 1990), including humans (Hochachka *et al.* 1996), and in agreement with those few animal studies that have examined this issue noninvasively in slow-twitch muscle (Kushmerick *et al.* 1992). In earlier studies (Matheson *et al.* 1991; Hochachka *et al.* 1991; Hochachka, 1994), the coupling patterns between ATP-demand and ATP-supply pathways in cardiac and slow-twitch muscles were described as ‘tighter’ than in fast-twitch muscles because large changes in ATP turnover rates could be sustained with modest (or immeasurable) changes in levels of these key high-energy phosphate metabolites.

#### Stability of metabolite concentrations during rate changes is general

The above results for human muscle are in no way unusual. Similar conclusions for the adenylates, phosphagen, P<sub>i</sub> and H<sup>+</sup> arise from studies of a wide assortment of animals as well as other human studies. These include invertebrates (Wegener *et*

*al.* 1991), fishes and other ectothermic vertebrates (Dobson and Hochachka, 1987), mammals and birds (see Hochachka, 1994, for references in this area). What is more, some of these studies have also analyzed many of the intermediates in specific ATP supply pathways, such as glycolysis (Dobson *et al.* 1988; Hochachka *et al.* 1991) and the Krebs cycle (Rowan and Newsholme, 1979); here too, changes in the concentrations of

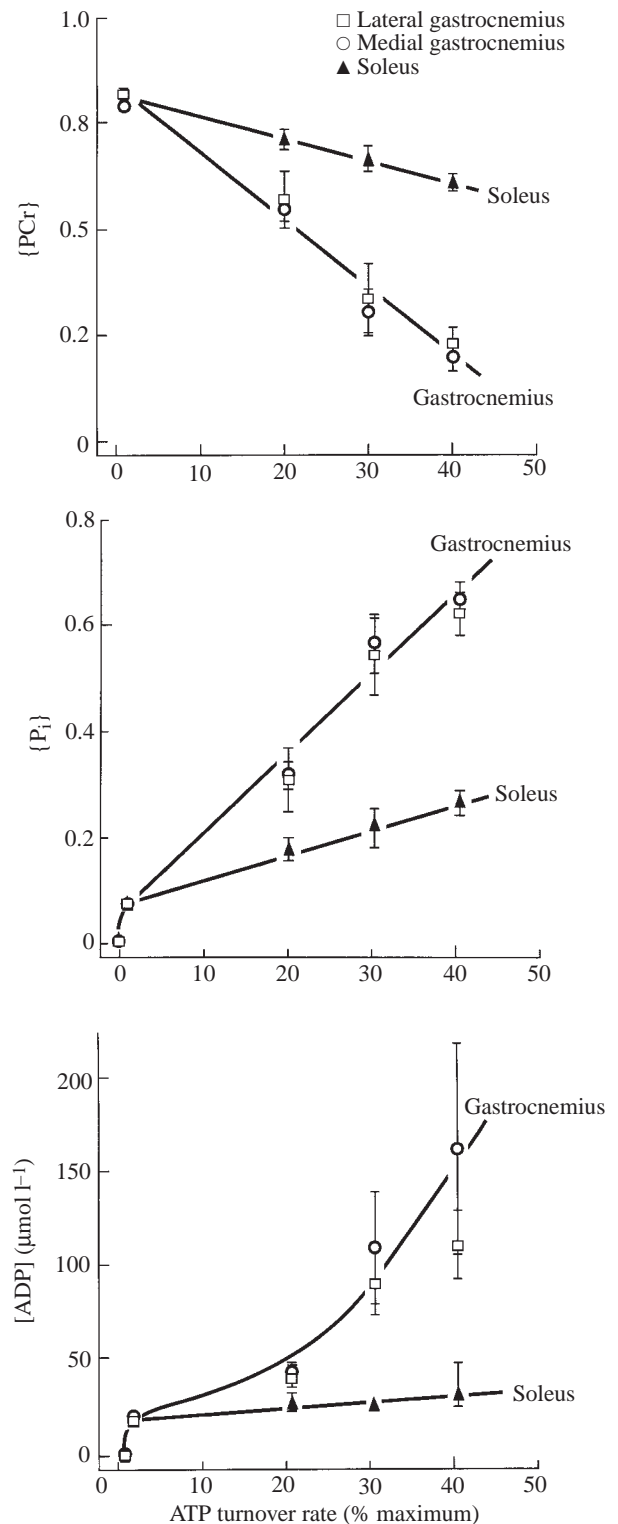


Fig. 2. (A) Change in muscle ATP turnover rate (as percentage of sustained maximum) plotted as the independent parameter *versus* phosphocreatine {PCr} (see text) as the dependent parameter. (B) Change in muscle ATP turnover rate (as percentage of sustained maximum) plotted as the independent parameter *versus* {P<sub>i</sub>} as the dependent parameter. At zero {P<sub>i</sub>}, the ATP turnover rate is assumed to be necessarily zero. (C) Change in muscle ATP turnover rate (as percentage of sustained maximum) plotted as the independent parameter *versus* [ADP] as the dependent parameter. At zero [ADP], the ATP turnover rate is again assumed to be necessarily zero. The concentrations of PCr and P<sub>i</sub> are given as a percentage of the sum of [PCr]+[P<sub>i</sub>]+[ATP]; the notations {PCr} and {P<sub>i</sub>} were introduced by Matheson *et al.* (1991) (unpublished data from P. S. Allen, G. O. Matheson, G. Zhu, R. S. Dunlop, T. Falconer, C. Stanely and P. W. Hochachka, in preparation; values are means ± S.E.M., N=4).

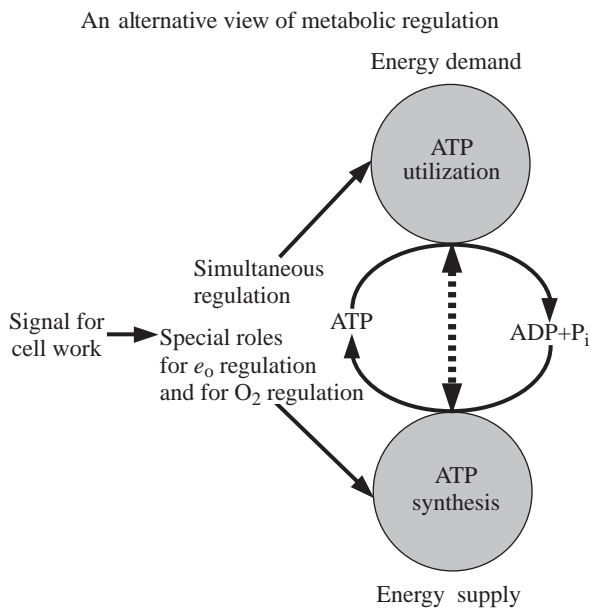


Fig. 3. Schematic diagram of an alternative model for integrating ATP demand and ATP supply pathways; in this view, control signals simultaneously activate both arms of the ATP cycle. Feedback regulatory mechanisms are assumed to supply fine tuning while  $e_0$ - (see text) and  $O_2$ -dependent mechanisms supply the coarse control. Based on Hochachka and Matheson (1992) and Hochachka (1994).

pathway intermediates are modest (0.5- to threefold) despite large changes in pathway fluxes that are simultaneously sustained by the working tissue.

The conclusions that emerge to this point therefore are (i) that [ATP] is almost perfectly homeostatic under most conditions (except under very extreme fatigue conditions) and (ii) that other intermediates in pathways of ATP supply or ATP demand are stabilized within less rigorously controlled concentration ranges. The latter may be termed a 'relatively' homeostatic condition, since the percentage changes in concentrations of intermediates are far smaller than the percentage changes in metabolic rates with which they correlate.

In tissues such as liver, where the difference between RMR and maximally activated metabolism is much more modest, the main model used to explain stable concentrations of adenylates (and other intermediates) at varying ATP turnover rates assumes coordinate control by  $Ca^{2+}$  of both ATP-supply and ATP-demand pathways (see McCormack and Denton, 1990, and literature therein). For muscle and heart, these kinds of mechanisms seem inadequate to account for the rate changes observed (Balaban, 1990). We have argued (Hochachka and Matheson, 1992; Hochachka, 1994) that the simplest model to account for these observations of unanticipated metabolic homeostasis assumes regulation of the concentrations of catalytically active enzymes in pathways of both ATP demand and ATP supply ( $e_0$  regulation); this would achieve changes in ATP turnover rates (Fig. 3) proportional to the  $k_{cat}$  (first-order rate constant for conversion of enzyme-substrate complex to enzyme-product complex) of the enzymes involved with no required change in substrate or product concentrations (see

Blum *et al.* 1990, 1991, for a possible example of this kind of regulation). Another model for enzymes which operate under near-equilibrium conditions assumes that very high catalytic capacities ensure sensitive 'high-gain' responses to small changes in substrate/product concentration ratios (see Betts and Srivastava, 1991; Hochachka, 1994, for references). Such near-equilibrium function of creatine phosphokinase (CPK) is the accepted explanation for the especially precise regulation of [ATP] during rate transitions – the traditional ATP 'buffering' role of CPK (Fig. 4). In any event, for models assuming key regulatory roles for pathway intermediates, the relative homeostasis of most metabolites consistently presents a serious problem: the percentage change in concentrations of putative regulatory intermediates is always less than the percentage change in flux required to match the change in ATP turnover rate. Put another way, the kinetic order is usually less than 1, too low to be 'driving' the observed flux or metabolic rate changes. The only metabolite which seems to be an exception is oxygen.

#### Role of oxygen delivery in metabolic regulation

The literature on how oxygen functions both as a substrate and as a potential regulator of metabolic rates of tissues is too large to review comprehensively here (see Hochachka, 1988; Hochachka *et al.* 1988). For working muscle, suffice to emphasize that numerous studies have found essentially 1:1 relationships between oxygen delivery and muscle work. For example, in recent studies (Arthur *et al.* 1992; Hogan *et al.* 1992) using a dog gastrocnemius preparation, we found such a relationship between oxygen delivery and work rate over an 18-fold change in ATP turnover rate. Later, Hogan *et al.* (1996) used the same preparation to analyze subtle submaximal work rate changes; these transitions were sustained with immeasurable change in phosphocreatine and ATP concentrations; presumably, therefore, concentrations of other metabolites were also stable. Yet through these transitions a 1:1 relationship

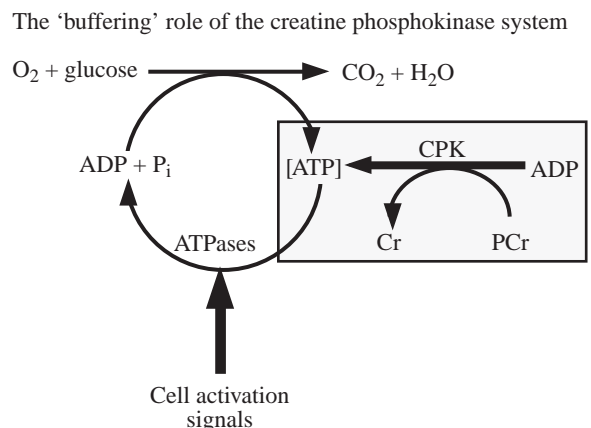


Fig. 4. Schematic diagram illustrating the 'buffering' role of creatine phosphokinase (CPK) in skeletal and cardiac muscle in which PCr is phosphocreatine and Cr is creatine. The diagram is simplified and does not include the possibility for localized CPK 'buffering' and 'transport' functions within the cell, for which there is convincing evidence (Bessman and Geiger, 1981; Wallimann *et al.* 1984, 1992).

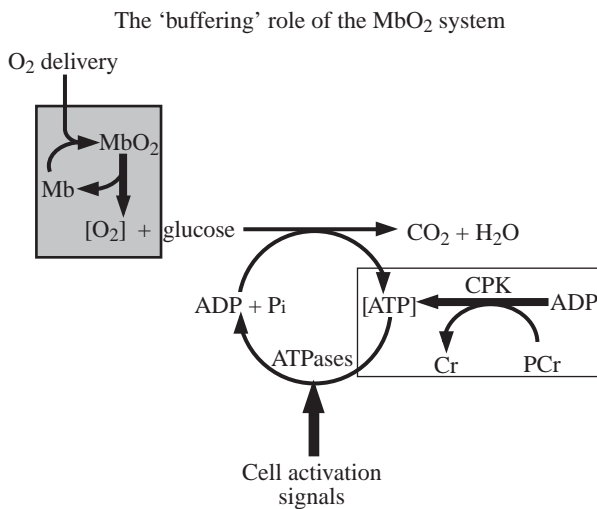


Fig. 5. Schematic diagram illustrating the 'buffering' role of myoglobin (Mb) in skeletal and cardiac muscle. Current data are consistent with earlier theoretical considerations in showing that percentage oxymyoglobin (MbO<sub>2</sub>) remains constant over broad ranges of O<sub>2</sub> flux to mitochondrial metabolism (see Jelicks and Wittenberg, 1995; Richardson *et al.* 1996). PCr, phosphocreatine; Cr, creatine; CPK, creatine phosphokinase.

between change in work and change in oxygen delivery was maintained. As emphasized, these kinds of results are qualitatively similar to those found in many other studies. That is why we and many others in the field accept that oxygen plays a key role in regulating change in ATP turnover (Hochachka, 1994). But how is the oxygen signal transduced within the cell? This issue, to which we shall now turn, remains unclear.

### Oxygen signal transduction in working muscle

Resolving the problem of how oxygen delivery translates into effects on metabolism within the cell requires information on intracellular oxygen concentrations. For most tissues, this key parameter has been simply too elusive and it thus remains unknown. Fortunately, in muscles, myoglobin (Mb) supplies a direct intracellular detector of oxygen concentration; because the reaction  $\text{Mb} + \text{O}_2 \rightleftharpoons \text{MbO}_2$  is always in equilibrium (McGilvery, 1983), with an apparent  $K_d$  of approximately  $0.1 \text{ mmol l}^{-1}$  ( $P_{50}$  of about 2–3 mmHg), measures of percentage MbO<sub>2</sub> supply a direct measure of intracellular oxygen concentration. Earlier attempts at such measurements with working muscle preparations relied almost exclusively upon near-infrared spectroscopy (see Guyton *et al.* 1996, for an unusual application of this technique in voluntarily diving Weddell seals). More recently, workers have used magnetic resonance spectroscopy (MRS) to take advantage of a histidine H being MRS-'visible' in deoxymyoglobin but being MRS-'invisible' in oxymyoglobin. This new technology for the first time supplies workers in the field with a noninvasive means for unequivocally 'detecting' the oxygenation state of Mb-containing muscles in different work and metabolic states. When applied both to working human skeletal muscles (Richardson *et al.* 1996) and to heart (Jelicks and Wittenberg, 1995), the same

striking observation arises: a relatively constant percentage oxymyoglobin despite large changes in work rate. What this means for working skeletal muscle is that percentage oxymyoglobin (and intracellular [O<sub>2</sub>]) remains constant up to the maximum sustainable aerobic metabolic rate of the tissue (Richardson *et al.* 1996). Just as creatine phosphokinase serves to 'buffer' ATP concentrations during changes in muscle work, so Mb serves to 'buffer' intracellular oxygen concentrations in different metabolic states (Fig. 5). In our context, under normoxic conditions, oxygen is thus perfectly homeostatic in the sense that its concentration is stable even while its flux to cytochrome oxidase can change by orders of magnitude.

To recapitulate, the situation arising from these new studies of oxygen and metabolic regulation can now be summarized as follows. First, because of the buffering role of Mb, oxygen concentrations are low (in the  $P_{50}$  or  $K_d$  range) and intracellular [O<sub>2</sub>] gradients must be quite shallow (this point is more fully discussed elsewhere; Connett *et al.* 1985; Gayeski and Honig, 1986). Secondly, the low intracellular [O<sub>2</sub>] is powerfully 'buffered' by Mb and remains essentially stable throughout large changes in work and metabolic rates. Nevertheless,  $\dot{V}_{\text{O}_2}$  and oxygen delivery are closely related, suggesting a key role for oxygen in metabolic regulation.

Given that it is oxygen delivery, not intracellular [O<sub>2</sub>], which correlates with work rate, the problem we are left with concerns the issue of how the oxygen signal 'gets through' to the machinery of cell metabolism. At this time, we admit that there is no widely accepted answer. To us, it seems necessary to postulate an oxygen-sensing system presumably located in the cell membrane (or even more distally) and signal-transduction pathways or mechanisms for 'telling' the cell metabolic machinery when to respond to the changing availability of oxygen (see Hochachka, 1994). However, the nature and even existence of such sensing and signal-transducing systems remain to be elucidated.

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