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Control of oxidative metabolism and oxygen delivery in human skeletal muscle: A steady-state analysis of the work/energy cost transfer function

(cardiac muscle/inorganic phosphate/phosphocreatine/nuclear magnetic resonance/metabolic disease)

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ABSTRACT **The concept of transfer function for organ performance (work output vs. biochemical input) is developed for skeletal and cardiac muscle under steady-state exercise conditions. For metabolic control by the ADP concentration, the transfer function approximates a Michaelis–Menten hyperbola. Variation of the work identifies metabolic operating points on the transfer function corresponding to ADP concentrations or to a ratio of inorganic phosphate to phosphocreatine that can be determined by phosphorus nuclear magnetic resonance. This operating point is characterized by the fraction (V/V_{max}) of maximal activity of oxidative metabolism in the steady state. This quantity appears to be useful in predicting the degree to which metabolic homeostasis is effective; poorly controlled metabolic states can readily be identified and are used in the diagnosis and therapy of metabolic disease in the organs of neonates and adults.**

Analytical biochemistry has great strengths in measuring the more stable components of cell bioenergetics, particularly ATP [as buffered by creatine kinase equilibrium in skeletal tissue, brain, and heart (1, 2)]. However, the more labile and indeed interesting components, phosphocreatine (PCr) and inorganic phosphate (P_i) are measured with significantly less accuracy for two reasons: (*i*) the breakdown of PCr during extraction in the interval between cessation of metabolism and assay and (*ii*) even more serious, the difficulty in distinguishing, by usual analytical techniques, the bound and free forms and the contents of different intracellular compartments (3).

Phosphorus NMR (P NMR) is selectively sensitive to the unbound form of cell metabolites and affords a wholly noninvasive approach to the study of metabolic control in the cytoplasmic compartment of cells and tissues (4, 5). P NMR can be used to obtain the relative concentrations of PCr, P_i, and ATP with rapidity and with significant accuracy (±10% in a 1-min scan). These concentration ratios are of great usefulness and importance in the study of metabolic control in animal models, neonates, and adults. Additional information is available when the absolute values of tissue concentrations of PCr and P_i are calculated from the value of ATP, and also creatine, as determined by analytical biochemistry [or prospectively by proton NMR (6, 7)]. When ADP plays its usual role as a regulatory metabolite, its concentration is maintained too low to be directly determined by NMR but can be calculated from the PCr/P_i value with appropriate assumptions. Under these conditions, NMR becomes a very useful tool because the principal elements of energy metabolism are determined and thermodynamic values may be estimated. As we shall discuss here, rates of oxidative metabolism relative to their maximal rates for the particular tissue conditions may be determined with significant accuracy particularly when P NMR data are used to include the effect of pH. We shall show how P NMR can be used, particularly in tissues stressed with hypoxia, for the prediction of stability, quasistability, and instability of oxidative metabolism in relation to the particular work load—i.e., functional ATPase activity.

Explanations of the response of body organs to ischemia and hypoxia have been offered from time to time (8) and most of these suggest that the primary event on the pathway to cell damage involves an acidosis. The effects may be catastrophic upon the functionality of both oxidative and glycolytic phosphorylation and upon ionic equilibria. A key question arises, namely, is there an intrinsic property of oxidative metabolism in a hypoxic/ischemic stressed tissue that renders it susceptible to a metabolic catastrophy with minimal acidosis and that may be exacerbated by greater acidosis? We term such a possibility a "kinetic failure" as opposed to an "acidotic failure." The kinetic failure occurs when the ATP synthesis by mitochondria fails to meet the needs of the functional ATPase and a steady state can no longer be maintained, and a "metabolic death" follows (9, 10). Thus, the instability of oxidative metabolism can be caused by its incapability for adequate metabolic work, which, as we describe below, can be evaluated by the PCr/P_i ratio. Furthermore, our analysis shows how failure of metabolic control can be predicted by P NMR and avoided by adjustment of the work load.

Control of Metabolism

The parameters relevant to control of oxidative metabolism include not only the delivery of ADP and P_i from the functional ATPase but also of oxygen from the capillary circulation and the delivery of substrate to form NADH as indicated by the following equation where the value of 3.0 is approximate (11).

$$3\ ADP + 3\ P_i + NADH + H^+ + \tfrac{1}{2}\ O_2 = 3\ ATP + NAD^+ + H_2O. \quad [1]$$

The choice between ADP and P_i, or indeed ATP, as control chemicals for the rate of Eq. 1 is immaterial insofar as the equations below are concerned, but P NMR has permitted a much more precise evaluation of this historically vexing question (12). A truism, often ignored, is that the steady-

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Abbreviation: PCr, phosphocreatine.

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state concentration of the control chemical must lie within the bounds of its Michaelis constant (K_m), others not controlling must lie above the K_m value. Thus, the simple and elementary P NMR observation of undetectable ADP and readily detectable P_i, *in vivo* in the human forearm (13), reinforces the arguments for the primary role of ADP in the control of oxidative metabolism *in vivo* (14, 15).

Control of metabolism by P_i was long advocated (16, 17) because analytical biochemistry showed metabolism linked variations of P_i in yeast cells (18). However, quantitative measurements *in vitro* have shown the K_m for control of respiration in isolated mitochondria by inorganic phosphate to be approximately 1 mM. Thus, the resting state 4 values for unattenuated P_i control *in vivo* should be below 1 mM in skeletal tissue. However, P NMR shows the resting state 4 values of P_i somewhat more than 1 mM, which significantly attenuates the role of P_i as a primary control. By way of contrast, the values of ADP concentration calculated from the P NMR data in the resting state 4 of skeletal tissue are a small fraction of K_m, consistent with ADP control of the respiration rate. In functional activity, in the transition from resting state 4 to the active state 3, the ATPase produces equal amounts of ADP and inorganic phosphate causing ADP to rise 50 times more in relation to its K_m than in the case of P_i, ensuring that the primary control will be that exerted by ADP.

Studies of isolated mitochondria showed that serial additions of increasing concentration of ADP resulted in a "Michaelis–Menten response" of respiratory activity with a half-maximal value of 20 μM (19). At the same time, the redox state of NADH as studied by our fluorometric method (20) responded similarly to ADP. By using this *in vitro* response of NADH to ADP, as an *in vivo* ADP indicator in stimulated muscles, both perfused and *in situ*, a similar sensitivity of mitochondria *in vivo* to ADP produced by ATP breakdown in single twitches was demonstrated (15, 20, 21). Thus, only a small fraction of the total ADP (bound plus free) as measured by analytical biochemistry was sensed by the mitochondria. P NMR results are especially relevant here since the freely tumbling form of the phosphate compounds is measured. When the P NMR creatine kinase are used to calculate the ADP concentration, it is found to be well below the total ADP as measured by analytical biochemistry and consistent with that estimated by fluorometry of mitochondrial NADH.

Metabolic Control Model

We have chosen a steady-state model since this simulates the physiological condition characteristic of life (22). In the feedback diagram of Fig. 1, ATP synthesis matches ATP breakdown by a tight feedback control loop. Activation of the functional ATPase to break down ATP into ADP and P_i [in most cases mediated by creatine kinase in the cytosol and at the mitochondrial membrane so that PCr, Cr, and P_i are the highly concentrated diffusable reactants (14)] produces ADP and phosphate which is translocated into the mitochondrial matrix to activate oxidative phosphorylation. The key feature of the system (Eq. 1) is that the ADP (or phosphate) concentrations regulate the rate of oxidative phosphorylation exactly to meet the needs of the functional ATPase so a homeostasis of the ATP level is obtained. At the same time, the level of ATP is temporally and spatially buffered by the creatine kinase equilibrium so that sudden changes of functional ATPase activity cannot cause wide swings of the rate of oxidative phosphorylation. As ATPase rates approach the maximal rate of oxidative phosphorylation (state 3), glycolysis, which is similarly activated by ADP and P_i, takes over and assumes an increasing proportion of the metabolic burden. Thus, for each level of functional activity, there will be

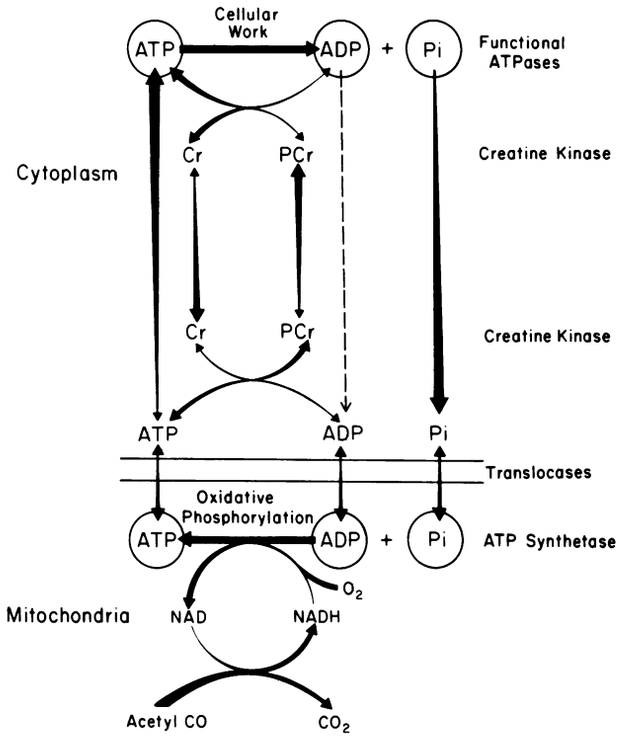

FIG. 1. Illustration of the feedback interactions of the cytosolic and mitochondrial compartments of muscle cell.

particular concentrations of the control chemical, ADP, and here we emphasize its value as calculated from the NMR data.

The Transfer Function

The function that relates work to the concentration of the control chemicals is termed the transfer function. There can be a characteristic transfer function for ADP, P_i, oxygen, NADH, or any of the parameters of Eq. 1 above. The intersection of the work or load line with the transfer function is termed the operating point. In any steady-state situation, the operating point is confined to move along the transfer function as the work varies. The transfer function is represented theoretically by the Michaelis–Menten relationship and can be calculated in terms of NMR parameters. We determine the transfer function *in vivo* from an arm exercise protocol.

The transfer function for ADP control is the Michaelis–Menten hyperbola:

$$\frac{V}{V_{max}} = \frac{1}{1 + \frac{K_m}{[ADP]}}. \quad [2]$$

V_{max} varies with pH in a typical bell-shaped curve symmetrical about the physiological pH (23). The NMR parameters can conveniently be substituted into Eq. 2 from the creatine kinase equilibrium.

$$PCr + ADP + H^+ \xrightarrow{K_{obs}} ATP + Cr \quad [3]$$

$$[ADP] = \frac{[Cr] \times [ATP]}{K_1 [PCr] \times [H^+]}. \quad [4]$$

For this discussion, we shall assume a constant pH of 7.1, a temperature of 37°C, a 1.0-mM magnesium concentration, and a 5-mM ATP concentration giving $K^+_{obs} = 132$ (1) and K_m

ADP = 20 μM (19, 24). The Michaelis–Menten hyperbola now becomes:

$$\frac{V}{V_{max}} = \frac{1}{1 + \left(\frac{0.53}{[Cr]/[PCr]}\right)}. \quad [5]$$

However, it is useful for P NMR spectroscopy to measure the ratio of PCr/P_i since this compensates for scale factor and other changes. Under our conditions of steady-state exercise, we observe that fits of data to this function are obtained (Fig. 2) when P_i/PCr is the recorded variable. Thus, P_i and Cr seem to be equivalent although the analytic values differ significantly. Thus, the substitution of P_i/PCr for Cr/PCr in Eq. 5 gives a very useful semi-empirical relationship:

$$\frac{V}{V_{max}} \approx \frac{1}{1 + \left(\frac{0.53}{[P_i]/[PCr]}\right)}. \quad [6]$$

Thus, the transfer function between work, V/V_{max}, and biochemical response P_i/PCr is expected to approximate a rectangular hyperbola with K_m 0.53. In order to test this relationship, we have studied muscle exercise performance over the several years (13, 25). V is readily varied by exercise in order to determine whether the simple equation fits the experimental data.

Steady-State Exercise Protocol

The subject exercises in a graded steady-state exercise, from rest to K'_m—i.e., P_i/PCr < 1.0—and thereby maintains an approximately constant pH. The work measured by an ergometer coupled to the exercised limb and the P_i/PCr value is measured by a surface coil placed upon the exercising muscle (13). This protocol contrasts with nonsteady-state exercise protocols from rest to the point of "fatigue," lactic acidosis, etc., and generally nonsteady-state conditions (26).

Experimental Results

In many studies of normal and diseased limbs, we have found a linear relationship between work and values of ≈1.0 for P_i/PCr. Eq. 6 suggests that the total relationship is hyperbolic. Here we present data over a wider range of exercise and illustrate two general types of transfer functions, hyperbolic and sigmoid. In Fig. 2, the work of a human arm of a well-trained yachtsman is varied from 15 to 35 J/min and the corresponding values of P_i/PCr increase from the rest value of 0.1 to a value slightly over 1. The form of the curve in this case approximates a rectangular hyperbola with a V_{max} of 52 J/min and a K'_m of 0.65 ± 0.06. This value of K'_m compares favorably with the theoretical value of 0.53 of Eq. 6. The profile of Fig. 2 was repeated after a further 3 months of training and found to be unaltered for this particular well-trained athlete. In the profile of Fig. 3 below, the slope and V_{max} have been shown to increase due to intense competitive activity in Olympic trials, but the shape of the transfer function was unchanged in three distinct intervals of study.

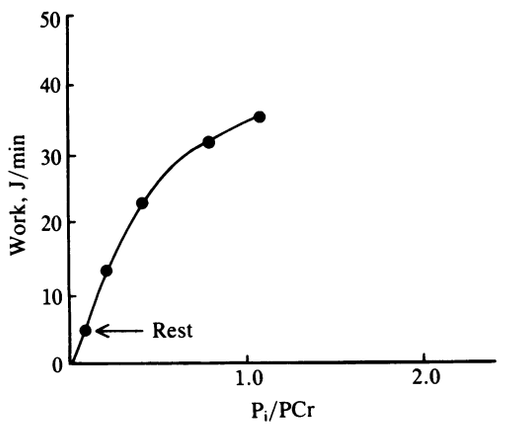

FIG. 2. A hyperbolic transfer function obtained for arm exercise (athletic young male).

If, for example, the velocity was related to the phosphate potential defined as ATP/ADP × P_i (27), then the ADP concentration of Eq. 2 is multiplied by the ratio of P_i/ATP. Since ATP is maintained constant by the creatine kinase equilibrium, the product of ADP and P_i gives a nonhyperbolic transfer function. For a total creatine (PCr + Cr) concentration of 30 mM, this leads to an equation nearly the same as Eq. 6. Data are similarly well fit.

Effect of Regulation of Oxygen Delivery on the Transfer Function

Fig. 3 represents an arm exercise study for an Olympic yachting aspirant where, in this case, the exercise protocol is started with the resting arm and includes values of V starting from 10% of V_{max} and increasing to over 50% of V_{max}. The transfer function is offset from a rectangular hyperbola up to a distinct discontinuity at P_i/PCr = 1 and thereafter approximates a hyperbola. One explanation of the difference between the transfer characteristics of Figs. 2 and 3 is that oxygen delivery is in excess in Fig. 2 and is adjusted to meet the varying tissue oxygen demands of Fig. 3. The choice between regulation and recruitment as determinants of sigmoidal transfer characteristics is simplified by our observation that the sigmoidal transfer function of Fig. 3 is linearized if the exercised limb is vasodilated from a previous exercise.

In order to calculate the effect of hyperemia upon the transfer characteristic, we assume a linear increase of oxygen delivery with P_i/PCr—i.e., $V_{max} = k\ P_i/PCr$. Substitution in Eq. 6 above gives

$$V = \frac{k\ P_i/PCr}{1 + \left(\frac{0.53}{P_i/PCr}\right)}. \quad [7]$$

We have calculated the values of V for the five points on the linear portion of the transfer function for k = 22 J/min; the approximate fit of this very crude hypothesis is encouraging.

In this transfer characteristic, ADP or P_i/PCr regulation of oxidative metabolism is complemented by regulation of O_2

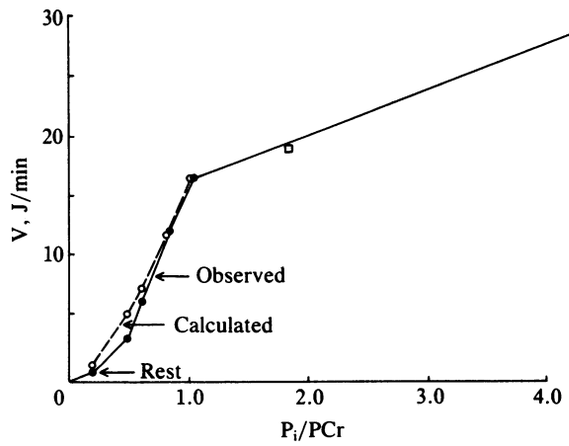

FIG. 3. A sigmoidal transfer function for arm exercise (athletic young female). V calculated for k = 22 J/min (○); V observed (● and □).

delivery to give a "tight" or "stiff" feedback control and a more precise homeostasis that minimizes the variation of P_i/PCr with work. At high P_i/PCr, increase of oxygen delivery is no longer possible and a transition to a hyperbolic characteristic occurs with a further activation of lactic acid formation. We may contrast the data of Figs. 2 and 3, one a rectangular hyperbola with a continuous transition to maximal work and lactic acidosis (28); the other a sigmoid with a mild lactic acidosis up to P_i/PCr = 1 and then a larger increase of lactic acid as the limit of the regulation of oxygen delivery is reached (29). A large class of transfer characteristics is sigmoidal as observed in more than 50 examinations of normal athletes.

Transfer Characteristics of Other Organs

The heart can be considered to be the organ of the body that is maximally adapted to endurance performance, in fact, for the lifetime of the individual. This may be especially true in the cardiac performance of "pursuit" type animals, such as dogs, compared with "sprint" type animals, such as cats. We have, therefore, selected the beagle dog as an example of an endurance performer and have made preliminary P NMR measurements of the transfer function of the cardiac tissue (Fig. 4). The NMR surface coil is implanted via a left thoracotomy and is glued directly to the surface of the left ventricle (30, 31). While the accurate measurement of heart work is difficult, we have employed the rate–pressure product as an approximation and increases of this quality by various levels of isoproterenol are plotted against the observed P_i/PCr values (Fig. 4). The values of P_i include a relatively small component due to the blood 2,3-bisphosphoglycerate and P_i as seen through the ≈1-cm thick ventricular wall by the 1.5-cm diameter surface coil. Thus, the variation of P_i may be even less than that shown. The transfer characteristic is very steep and a likely explanation is a coupling of V_{max} and PCr/P_i according to Eq. 7. In the heart, the set point for regulation is at a PCr/P_i value of 5:1, or one third of V_{max}. This extremely conservative operating point provides a high phosphate potential and permits endurance performance over a wide range of values of V without reaching saturation of oxygen delivery and oxidative metabolism.

Preliminary experiments on newborn lambs and cats suggest that the transfer functions are less steep than that observed in Fig. 4, presumably because of lack of maturation or lack of need for endurance performance in the mature animal (30).

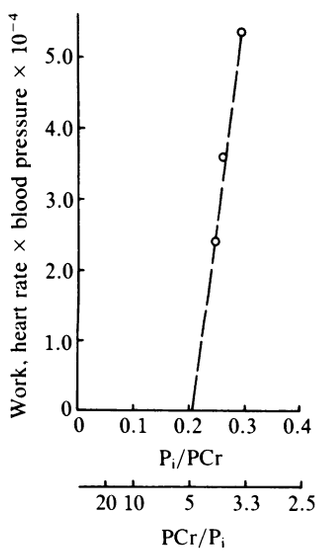

FIG. 4. A transfer characteristic for the left ventricle of an anesthetized beagle dog from an implanted surface coil.

The Transfer Characteristics for Oxygen and ADP Control

Oxygen delivery limitation controls the V_{max} of the transfer function and can be represented graphically as shown here or mathematically as we have shown for alcohol dehydrogenase (32). The transfer characteristic for oxygen delivery is also hyperbolic as determined experimentally (33). The value of the Michaelis–Menten affinity for oxygen control is given by the expression $K_mO_2 = k_3/k_1$, where k_3 is the turnover number (<5 sec^{-1} in state 4 to 50 sec^{-1} in state 3) and k_1 is the second order velocity constant for the reaction of cytochrome oxidase with oxygen (5×10^7 M^{-1} sec^{-1}) (34). Thus, if we take an activity double the rest level (≈10/sec), the K_m is 2×10^{-7} M or 0.1 torr (1 torr = 133.3 Pa). The hyperbolic transfer function is displayed on the right-hand side of Fig. 5. If the tissue oxygen concentration available to the mitochondria is 0.15 torr, slightly above K_m, the arterial oxygen concentration is of course much greater (>20 torr) because of tissue oxygen gradients. A concentration of 0.15 torr corresponds to 70% of maximal rate on the oxygen transfer characteristic (point A_3) and sets the asymptote of the transfer characteristic for ADP control V_{max} (left portion of the graph). For a given work load, A_w, the operating point as defined above is at point A_3, giving values of ADP and P_i/PCr of 25 μM and 0.66, respectively. Under these conditions, the system is stable and well controlled.

Effects of Hypoxia

Fluctuations in the oxygen delivery to tissue will move the operating point for oxygen delivery along the rectangular hyperbola for oxygen control resulting in a different V_{max} for the ADP transfer function; for example, there is a 50% decrease of the oxygen concentration from point A to point B, the maximum of the ADP transfer function will be lowered proportionally, giving the transfer characteristic B_3. The operating point now moves from PCr/P_i of 1.5 to 2.2, and ADP from 25 to 77 μM.

Unstable or Poorly Controlled Metabolic States

The operating point is now at a quasistable or poorly uncontrolled point since further diminution of the oxygen delivery moves point B_3 to the left in the oxygen control diagram and to the right in the ADP control diagram.

At high levels of activation, [ADP] > K_m, the transfer function is nonlinear leading to an unbalanced activation of glycolysis and respiration. Lactate will accumulate at a rate equal to the imbalance between glycolytic and oxidative metabolism. Muscle lactate efflux is proportional to the difference in intracellular and blood lactate levels and intracellular lactate levels will assume a steady-state level approximately proportional to the imbalance in glycolytic and mitochondrial rates.

Positive feedback may also occur: lactic acidosis that will further deteriorate the ADP control transfer function by inhibiting both the efficiency and the rate of oxidative phosphorylation (23), leading to loss of metabolic control, that is, a loss of PCr, ATP, cell ion gradients, and membrane integrity—cell death. The rationale of classical therapeutic procedures are now explained; they move the operating point to a lower P_i/PCr by (*i*) restoring the oxygen concentration, (*ii*) decreasing the work load (as in skeletal tissues), and (*iii*) proton pumping. Although. the beneficial effect of β blockade in humans with ischemic heart disease is to limit cardiac work and, hence, metabolic demand decreases of work could be self-defeating for the heart and is rarely possible for the adult brain. If the brain ATPase rate exceeds the ATP synthesis rate, a "metabolic brain death" occurs as the oper-

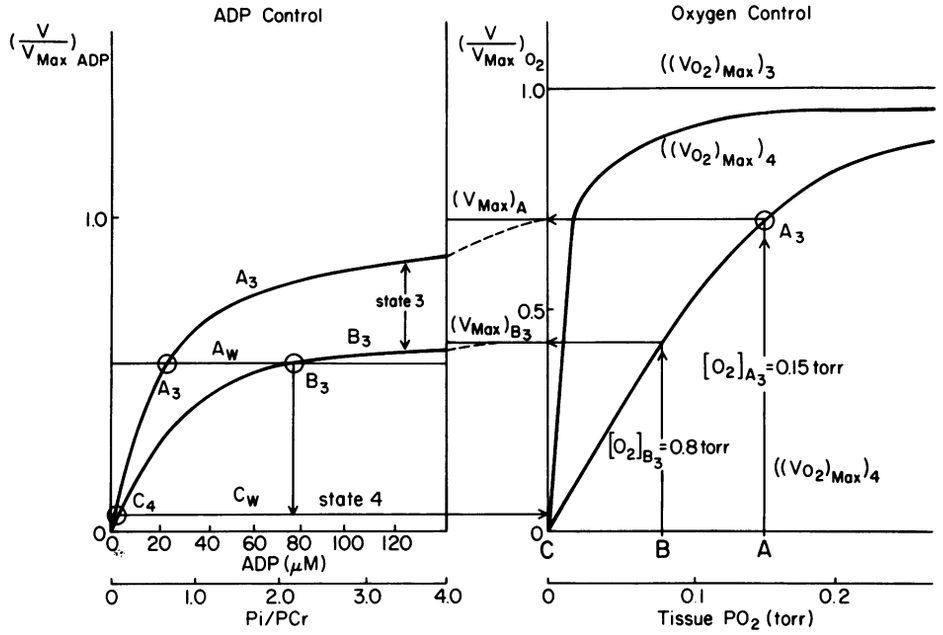

FIG. 5. Metabolic control in normoxia, hypoxia, work, and rest. Calculated transfer functions for Michaelis–Menten mechanisms for oxygen control (right), and ADP control (left) at constant pH and variable oxygen delivery and work load.

ating point moves to higher ADP and P_i/PCr levels. Furthermore, the metabolic load line is likely to rise due to the high extracellular potassium concentrations causing near maximal rate of the brain ATPase activity. Similarly, with the heart, hypoxia may cause a tachycardia raising the load line of an already stressed cardiac tissue. In this case, cardiac arrest will cause ischemia and lower the tissue PO_2 drastically. Thus, the two key organs of the body have metabolic feedback loops that are poorly controlled in metabolic stress and the motion of the operating points to unstable regions may lead to irreversible loss of control and cell death. ^{31}P NMR can be a key instrument in the detection of and, where possible, the stabilization of such poorly controlled states in the body organs by the therapeutic control of the three parameters mentioned above.

A further aspect of these transfer functions discussed elsewhere (35) is that a decrease of the work from the active state 3 value towards the resting state 4 value, as for example, upon cessation of work in the exercised arm has two beneficial effects: (*i*) the load line drops to <10% of V_{max}, causing a rise of PCr/P_i to a stable value and (*ii*) this load line permits stable operation at much lower value of tissue pO_2 because the K_mO_2 will be left shifted in proportion to the decrease of V (35). This "saving" of the tissue by the state 3 → state 4 transition may be employed in many cases as a therapeutic procedure and is readily observed in diving animals [termed metabolic arrest (36)] and may indeed be the case in neonates. It is an approach that merits a thoughtful future development.

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