Metabolic control over the oxygen consumption flux in intact skeletal muscle: in silico studies

Piotr Liguzinski and Bernard Korzeniewski
Faculty of Biotechnology, Jagiellonian University, Crakow, Poland

Submitted 17 February 2006; accepted in final form 2 June 2006

IN SKELETAL MUSCLE during mechanical work, ATP is used mostly by actomyosin-ATPase and Ca\(^{2+}\)-ATPase. Three main processes are responsible for the resynthesis of ATP from ADP during muscle exercise. Creatine kinase (CK) is the main ATP producer during the first (tens of) seconds of exercise. Anaerobic glycolysis delivers significant amounts of ATP during the initial stage of exercise and during short-term high-intensity exercise. Oxidative phosphorylation is the main source of ATP in most muscles under most physiological conditions. The general scheme of the oxidative phosphorylation system in skeletal muscle is presented in Fig. 1. In addition, the fourth process that leads to ADP consumption, and, to a less extent, ATP resynthesis, is AMP deamination taking place during heavy exercise and/or hypoxia (30).

During rest-to-work transition in skeletal muscle, a \(>100\)-fold increase in energy demand (ATP usage) may take place. The rate of ATP synthesis must very quickly match the rate of ATP utilization to avoid a very rapid ATP exhaustion, termination of exercise, or even cell death. Three main mechanisms responsible for adjusting the rate of ATP production by oxidative phosphorylation to the current energy demand have been postulated.

With the first mechanism, which will be called the output-activation mechanism, only ATP usage (output of the system) is directly activated by calcium ions during muscle work, whereas oxidative phosphorylation is activated only indirectly, through negative feedback involving increase in \([\text{ADP}]\) and \([\text{Pi}]\). It was originally postulated by Chance and Williams (4, 5) that the dependence of the respiration rate (\(V_{\text{O2}}\)) on ADP concentration is hyperbolic (first order at low ADP concentrations). Jeneson et al. (19) modified this proposal and postulated that the mechanistic \(V_{\text{O2}}/[\text{ADP}]\) dependence is much steeper, at least second order. The output-activation mechanism is presented in Fig. 2A.

The discovery of the activation in vitro of the "key" tricarboxylic acid (TCA) cycle dehydrogenases (pyruvate dehydrogenase, isocitrate dehydrogenase, 2-oxoglutarate dehydrogenase) by calcium ions prompted several authors to postulate that substrate dehydrogenation (input of the system) is activated in parallel with ATP usage (input/output-activation mechanism) (13, 41). However, with this proposal oxidative phosphorylation is still activated only indirectly, via an increase in \([\text{ADP}]\) and \([\text{Pi}]\) and/or increase in the NADH/ NAD\(^+\) ratio. The input/output-activation mechanism is presented in Fig. 2B.

Theoretical studies by means of the computer model of oxidative phosphorylation in skeletal muscle mitochondria and intact skeletal muscle developed previously (35, 36) led to the conclusion that only a direct activation by some cytosolic factor of all oxidative phosphorylation enzymes is able to account for large changes in \(V_{\text{O2}}\) and ATP turnover accompanied by only very moderate increase in ADP concentration during rest-to-work transition in skeletal muscle (26–29). This mechanism, called the each-step-activation mechanism or parallel-activation mechanism is presented in Fig. 2C. Within this mechanism the feedback regulation via \([\text{ADP}]\) and \([\text{Pi}]\) plays only a minor, fine-tuning role in intact skeletal muscle in vivo (26, 29) and essentially no role in intact cardiac muscle (33).

It has been shown that the each-step-activation mechanism is able to explain many, apparently unrelated, kinetic properties of oxidative phosphorylation in skeletal muscle (26–29, 37). These properties comprise the relative stability of \([\text{ADP}]\), \([\text{Pi}]\), phosphocreatine/creatine (\(\text{PCr}/\text{Cr}\)), NADH/NAD\(^+\), and pro-
tonomotive force (Δp) during rest-to-work transition (26, 27), the much steeper phenomenological VO₂/[ADP] relationship in intact skeletal muscle than in isolated muscle mitochondria (26, 27), the two to five (to seven) times greater maximum oxygen consumption in intact human skeletal muscle than in isolated mitochondria, skinned fibers, or muscle homogenate when scaled for the amount of mitochondrial proteins (36, 44, 52), the increase in the relative slope of the phenomenological VO₂/[ADP] relationship as a result of muscle training (37), the greater VO₂ at a given [ADP] and amount of mitochondrial protein in trained muscle than in untrained muscle and in untrained muscle than in hypothyroid muscle (29), the asymmetry of the half-transition time for [PCr] between the on transient (rest-to-work transition) and off transient (work-to-rest transition) (29), and the PCr recovery overshoot (29, 39).

In addition, there is a great variability of the kinetic properties of oxidative phosphorylation in different muscles and various experimental conditions (29). To explain this variability, it was proposed that the intensity of the each-step activation is highest in oxidative skeletal muscle, medium in glycolytic skeletal muscle under physiological conditions, and low or absent in perfused glycolytic muscle stimulated electrically (29) (therefore, in the last case, the output-activation mechanism predominates). Recently, it was also postulated (33) that the each-step-activation mechanism is responsible for the almost perfect stability of several oxidative phosphorylation metabolites (ADP, PCr, P_i, NADH) between steady-states with very different work intensities and oxygen consumption rates (2, 14, 21, 50). The differences in the regulation of oxidative phosphorylation between skeletal muscle and heart are discussed in Ref. 33. Generally, the idea of the each-step activation in both skeletal muscle and heart seems to be well founded.

As to glycolysis, it also has been postulated that the regulation of this process by intermediate metabolites ADP, AMP, ATP, P_i, NADH, and NAD^+ (plus activation of glycogen phosphatase by calcium ions) is not sufficient to account for the kinetic behavior of this pathway in vivo (7) (for instance, ATP supply by anaerobic glycolysis is not significantly activated during anoxia and muscle recovery, when [ADP], [AMP] and [P_i] are elevated). In particular, it has been demonstrated that a direct activation of glycolysis by some external cytosolic factor is necessary to explain the large increase in the glycolytic flux taking place during transition from rest to heavy exercise in skeletal muscle (32).

Frequently the term “regulation of some biochemical pathway” is referred to the mechanism by which the metabolite flux through the pathway is changed in response to some external signal/disturbance. On the other hand, the term “control of the flux” may be attributed to the potential strength with which a given enzyme concentration/activity determines the flux through the entire system (28). The best means of describing the extent to which a given enzyme (or process or metabolic block) controls the metabolite flux through a given metabolic pathway is Metabolic Control Analysis (MCA) (9, 15, 20).
According to this theoretical approach, no enzyme determines the flux uniquely (there are no so-called “rate-limiting” steps). The contribution of particular enzymes to the control over the metabolite flux is quantified by a parameter called the flux control coefficient. It is defined as the ratio of the relative change in a given flux caused by a small relative change in the enzyme concentration/activity to this change itself. For enzyme \( i \) and flux \( J \) we have the following expression:

\[
C_{E_i}^J = \frac{\partial J/J}{\partial E/E_i}
\]  

(1)

where \( \partial J/J \) is a relative change in flux and \( \partial E/E_i \) is a small relative change in enzyme \( i \) concentration/activity. The flux control coefficient is a global parameter; it depends on the properties of the whole system and not only on the features of a given enzyme. The pattern of control in a given system, determined by the distribution of metabolic control among different enzymes, is different in different steady states. The values of flux control coefficient of all enzymes in a given pathway sum up to unity (the so-called summation property).

Flux control coefficients can be determined in the experimental way, for instance, by titration of particular enzymes with specific inhibitors, or calculated using appropriate kinetic models of biochemical pathways. It is worthwhile to emphasize that although according to MCA there are no “entirely rate-limiting steps” (the value of the flux control coefficient is never exactly equal to 1), in many situations some steps can be “practically rate-limiting” (the value of the flux control coefficient is very close to 1). This concerns, for instance, the control exerted by ATP usage over the ATP turnover flux (compare Figs. 5B and 6B).

It has been demonstrated (12) in the experimental way that in isolated liver mitochondria, in state 4 (no ATP production, high ATP/ADP) the proton leak through the inner mitochondrial membrane is the main step controlling the oxygen consumption flux, in state 3 (maximal ATP production, low ATP/ADP), the entire control is distributed more or less evenly among particular oxidative phosphorylation complexes, and in the intermediate state 3.5 (medium ATP production, medium physiological ATP/ADP) most of the control is kept by the artificial ATP usage system (hexokinase + glucose). Also in skeletal muscle mitochondria, in state 3 the control is shared by all oxidative phosphorylation complexes (40). A similar distribution of metabolic control was generated by the computer model of oxidative phosphorylation in isolated mitochondria developed by Korzeniewski and Mazat (35). In fact, the model was able to reproduce the whole inhibitor titration curves for particular complexes, and thus the model was validated for large-scale changes in fluxes and metabolite concentrations (35). In calcium-stimulated skinned skeletal muscle fibers at 80% of maximal (state 3), activation of ATP usage a pattern of control intermediate between state 3.5 and state 3 in isolated mitochondria was observed: half of the control was at actomyosin-ATPase, whereas the remaining half was shared by mitochondrial enzymes (57).

Jensen et al. (18) calculated, based on their experimental data, the flux control coefficients for ATP usage (by actomyosin-ATPase and calcium-ATPase) and ATP production (by oxidative phosphorylation) in an electrically stimulated glycolytic muscle. They concluded that, as in isolated mitochondria in state 3, at higher ATP turnovers (stimulation frequencies >3 Hz) the control over the ATP turnover flux is shifted from ATP consumption to ATP production, and therefore the latter becomes limiting for the flux. However, as discussed above and in Ref. 29, in electrically stimulated glycolytic skeletal muscle there is probably no each-step activation, and therefore only the output-activation mechanism (that is present in isolated mitochondria) is responsible for adjusting the rate of ATP production to the current energy demand in this case.

The aim of the present theoretical study is to analyze the effect of the each-step-activation (parallel activation) mechanism that was postulated to take place in oxidative and glycolytic skeletal muscle under physiological conditions (neurally stimulated muscle in vivo) (29) on the distribution of control over the oxygen consumption flux among ATP usage, substrate dehydrogenation, and particular oxidative phosphorylation complexes at different work intensities. It is demonstrated that each-step activation allows maintenance of more or less constant values of the flux control coefficients of particular steps over a very broad range of energy demand, and therefore ATP usage may remain the main controlling step even at very high ATP turnovers. It is also shown that at low oxygen concentrations, the oxidative phosphorylation system (in particular, cytochrome oxidase) takes over most of the control and becomes limiting for the ATP turnover and oxygen consumption flux. Overall, the present in silico study strongly suggests that under physiological conditions, the availability of oxygen, and not potential capacity of oxidative phosphorylation for ATP synthesis, may determine the maximal respiration rate, ATP turnover, and mechanical work intensity in oxidative skeletal muscle.

THEORETICAL PROCEDURES

The previously developed computer model of oxidative phosphorylation in intact skeletal muscle (36) was used in the present theoretical studies. The following enzymes, processes, and/or metabolic blocks are taken into account explicitly within the model: substrate dehydrogenation (hydrogen supply to the respiratory chain including TCA cycle, glycolysis, glycerolysis, glucose transport, fatty acid β-oxidation, fatty acid transport), complex I, complex III, complex IV (cytochrome c oxidase), proton leak, ATP synthase, ATP/ADP carrier, phosphate carrier, adenylate kinase, creatine kinase, and ATP usage. The dependences of the rates of particular reactions, processes, and/or metabolic blocks on different metabolite concentrations are described by appropriate kinetic equations. For instance, the rate of the reaction catalyzed by cytochrome oxidase (complex IV) \( v_{C4} \) is expressed as follows:

\[
v_{C4} = k_{C4} \cdot a^{2+} \cdot c^{2+} \cdot \frac{1}{1 + K_{mO}/[O_2]}
\]  

(2)

where \( k_{C4} \) is the rate constant, \( K_{mO} \) is the mechanistic Michaelis-Menten constant of cytochrome oxidase for oxygen, and \( a^{2+} \) and \( c^{2+} \) are concentrations of the reduced forms of cytochrome \( a_1 \) and cytochrome \( c \), respectively. The time variations of the metabolite concentrations that constitute independent variables (NADH, ubiquinol, reduced form of cytochrome \( c \), \( O_2 \), internal protons, internal ATP, internal \( P_i \), external ATP, internal phosphate, cytochrome oxidase, etc.) are described by appropriate kinetic equations.
external ADP, external $P_i$, external protons, and PCr) are expressed in the form of a set of ordinary differential equations. For instance, the differential equation for the reduced form of cytochrome $c$ has the following form:

$$ \frac{dc^{-2+}}{dt} = (v_{c3} - 2 \cdot v_{c4}) \cdot 2 \cdot R_{cm}$$

where the rate of change of the concentration of the reduced form of cytochrome $c$ in time $dc^{-2+}/dt$ is equal to the difference between the rate of its production by complex III ($v_{c3}$) and the rate of its consumption by complex IV ($v_{c4}$) (described by Eq. 1); $2 \cdot R_{cm}$ describe the stoichiometry (2 electrons for 1 NADH, 4 electrons for 1 O$_2$) and the ratio of cell volume to mitochondria volume, respectively. Of course, in a steady state, no changes in metabolite concentrations take place, because the rates of production and consumption of each metabolite are exactly equal. The other (dependent) variable values (other metabolite concentrations, thermodynamic forces) are calculated from the independent variable values. The set of differential equations is integrated numerically. In each iteration step, new values of rates, concentrations, and other parameters are calculated on the basis of the corresponding values from the previous step. The Gear procedure is used for numerical integration, and the simulation programs are written in the FORTRAN programming language. The complete description of the model is located on the web site http://awe.mol.uj.edu.pl/~benio/.

The model has been validated for a broad range of different parameter values and system properties in both isolated mitochondria and intact skeletal muscle and heart (24, 26, 28, 29, 33, 36). In the context of the present study, particularly important is the fact that the model reproduces correctly the inhibitor titration curves and flux control coefficients for particular oxidative phosphorylation complexes in state 3 in isolated mitochondria, where oxidative phosphorylation is saturated, and the metabolic control is distributed more or less uniformly between different enzymes (35).

In the first part of the study, the dependence of fluxes, metabolite concentrations, and flux control coefficients on energy demand for constant saturating oxygen concentration (240 $\mu$M) were investigated. In computer simulations, the energy demand (rate constant of ATP usage) was increased $n$ times in relation to the resting state; in subsequent simulations, $n$ increased by 1, from 1 (no activation) to 100. In the case of the output-activation mechanism, only ATP usage was directly activated. In the case of the each-step-activation mechanism, all oxidative phosphorylation complexes and substrate dehydrogenation were directly activated in parallel with the activation of ATP usage. The direct activation of particular oxidative phosphorylation complexes was equivalent to an increase in the rate constants (or maximal velocities) of these steps. High intensity of the each-step activation was used; this means that all steps in the ATP-producing block were activated $n^{0.4}$ times when ATP usage was activated $n$ times. The power coefficient equal to 0.4 was chosen to ensure that a large relative increase in $V_{O_2}$ during rest-to-work transition is accompanied by only a moderate relative increase in [ADP]; if this coefficient equals zero, then the phenomenological $V_{O_2}/[ADP]$ relationship becomes near hyperbolic (first order at low ADP concentrations), and if it is much higher than 0.4, then the relative increase in [ADP] becomes very small. In each simulation, the system stabilized at a new, active steady state that was verified using an appropriate numerical procedure, checking that the rates of changes in variable values were below some assigned, very low rate. The fluxes and metabolite concentrations in the active steady state were recorded, and the values of flux control coefficients were calculated according to Eq. 1. Rate constants/maximal velocities of particular steps were increased by 0.01%, and the resultant relative changes in the oxygen consumption and ATP turnover flux between the original and the new steady state were recorded. The summation theorem of MCA was used to validate the calculated flux control coefficients. The maximal error for the sum of flux control coefficients was < 0.001.

In the second part of the study, the dependence of fluxes, metabolite concentrations, and flux control coefficients on oxygen concentration for relative energy demand of 100 was investigated. Simulations were performed, assuming the each-step-activation mechanism (parallel activation of all oxidative phosphorylation complexes $10^{0.4}$ times in the active state). [O$_2$] ranged from 240 to 0.01 $\mu$M; in each subsequent simulation, [O$_2$] was lower by 3% in relation to the previous simulation. For each oxygen concentration, a new steady state was achieved; fluxes and metabolite concentrations were recorded, and flux control coefficients were calculated as discussed above.

Some other models of oxidative phosphorylation in skeletal muscle have been proposed. The model developed by Meyer (42), involving a simple linear dependence of ATP production by oxidative phosphorylation on [Cr], represents the output-activation mechanism and therefore is applicable only to perfused glycolytic muscle stimulated electrically (see text above and below, as well as Ref. 29). In addition, the linear phenomenological dependence on [Cr] can be derived from the more mechanistic near-hyperbolic dependence of oxidative phosphorylation on [ADP] (23, 36). The model developed by Connett and colleagues (6, 7) is also very phenomenological (it simply relates [ATP], [ADP], [AMP], [P$_i$], [PCr], and [Cr] to each other at different energy demands via the creatine kinase and adenylate kinase equilibrium) and implicitly assumes the output-activation mechanism. Both models treat oxidative phosphorylation as a black box, and therefore they do not analyze the internal properties of the system and, in particular, do not calculate the flux control coefficients of particular enzymes. They ignore the proton leak through the inner mitochondrial membrane and therefore do not distinguish between the oxygen consumption flux and ATP turnover flux. For all these reasons, although the discussed models provided a better understanding of several quantitative properties of oxidative phosphorylation in skeletal muscle, they could be used for only a limited set of theoretical studies.

THEORETICAL RESULTS

Figure 3 compares the simulated values of $V_{O_2}$ and metabolite concentrations at different energy demands (rate constants of ATP usage) for the output-activation mechanism (A) and the each-step-activation mechanism (B). The energy demand is expressed as a multiplicity of the rate constant of ATP usage at rest (the resting ATP usage equals 0.73 mM/min; its contribution to the respiration rate at rest is ~40%, with the remaining 60% being due to proton leak). One can see that in the case of output activation, the oxygen consumption increases linearly
with ATP demand up to the relative energy demand of about 30 and then remains constant (ATP production by oxidative phosphorylation becomes saturated). The maximal \( V_\text{O}_2 \) reaches 3.7 mM/min. This value corresponds well with the maximal oxygen consumption in isolated mitochondria, skinned fibers, and muscle homogenate (when recalculated for the amount of mitochondrial proteins) but is much smaller than the maximal \( V_\text{O}_2 \) in intact skeletal muscle, amounting to 7–16 (to 26) mM/min (1, 3, 36, 44, 52). On the other hand, each-step activation allows a linear increase in \( V_\text{O}_2 \) in the whole simulated range of ATP demand. The \( V_\text{O}_2 \) reaches >12 mM/min at a relative energy demand of 100 (the maximum value shown in Fig. 3) and can increase much more (up to 30 mM/min for [PCr] = 1 mM or up to 38 mM/min for very low [PCr]) at higher energy demands (not shown).

One may address the question as to how it is possible to maintain a steady state in the case of the output-activation mechanism when the relative energy demand is much greater than 30 and oxidative ATP production becomes saturated (see Fig. 3A). However, one must bear in mind that relative energy demand is equivalent to the rate constant of ATP usage and not to the rate of ATP usage itself. The latter parameter depends not only on the rate constant but also on [ATP]. When [ATP] drops much below the Michaelis-Menten constant of ATP usage for ATP (equal to 150 \( \mu \text{M} \) within the model), the rate of ATP usage may remain constant even if energy demand increases (the decrease in [ATP] compensates exactly the increase in the rate constant of ATP usage).

When the energy demand (rate constant of ATP usage) increases, the metabolite concentrations are much more constant in the case of the each-step-activation mechanism than in the case of the output-activation mechanism. In the latter case (Fig. 3A), metabolite concentrations change significantly up to the “critical” relative energy demand of 30; [PCr] drops to zero, whereas [Cr] and [P] rise to the maximal values (35 mM and 38 mM, respectively). [ATP] is relatively constant up to the relative energy demand of 27 because of a high ATP/ADP ratio and then begins to fall to zero. [ADP] increases to 1.6 mM and then decreases, whereas [AMP] starts to increase; this is caused by the reaction catalyzed by adenylate kinase. [ATP] decreases below the \( K_\text{m} \) constant of ATP usage for ATP, and therefore a further increase in the rate constant of ATP usage does not cause a further acceleration of ATP hydrolysis.

In the case of the each-step-activation mechanism, much more moderate changes in metabolite concentrations with an increased energy demand take place. There is no critical point at which concentrations achieve zero or maximal values (in fact, this critical point is present at much higher values of ATP demand than 100; see above and DISCUSSION). [ATP] is essentially constant in the whole range of energy demand analyzed. [AMP] remains at very low values, whereas [ADP] increases -10-fold between an energy demand of 1 (rest) and an energy demand of 100, from 7 to 68 \( \mu \text{M} \). [Cr] and [P] increase to 24 and 18 mM, respectively, whereas [PCr] decreases to 11 mM. At an energy demand of 100 there is still some “reserve” of ATP and PCr, and [ADP] also does not reach the maximal value encountered in intact skeletal muscle (over 100 \( \mu \text{M} \)). Therefore, a further increase in energy demand (above 100) can still cause a further increase in \( V_\text{O}_2 \) and ATP turnover, as discussed above. It is worthwhile to emphasize that the each-step-activation mechanism allows a much better ATP homeostasis at varying energy demand than the output-activation mechanism.

It seems to be interesting to compare what factors cause the increase in \( V_\text{O}_2 \) brought about by an increase in ATP demand in the output-activation mechanism and the each-step-activation mechanism. There are four potential signals transducing this stimulation of \( V_\text{O}_2 \): increase in [ADP], increase in [P], decrease in [ATP], and direct each-step-activation. Figure 4 presents the simulated contribution of these signals for both mechanisms at different energy demands. The increase in \( V_\text{O}_2 \) accompanying an increase in relative ATP demand by 0.01 is shown. The increase in \( V_\text{O}_2 \) caused by a given signal (S) is a product of two things: relative change in this signal intensity when relative ATP demand increases (\( dS \) for an increase in relative ATP demand by 0.01) and the sensitivity of \( V_\text{O}_2 \) to this signal intensity (\( dV_\text{O}_2/dS \)). In computer simulations, the values of \( dS \) for particular signals and the total \( dV_\text{O}_2 \) when the system passed from an old to a new steady state because of an increase in relative energy demand by 0.01 were recorded first. Next, the old steady state was restored and the “new” value of one signal S was set, while “old” values of the remaining signals were kept. The increase in \( V_\text{O}_2 \) (\( dV_\text{O}_2 \)) caused by a change in S was recorded. This procedure was repeated for all signals. Of course, the total increase in \( V_\text{O}_2 \) is the sum of the increases caused by particular signals.
It can be seen that in the output-activation mechanism (Fig. 4A), the stimulation is mostly, 80–90%, transduced via an increase in [ADP], with the remaining 10–20% being due to an increase in [Pi]. When the oxidative phosphorylation system is almost saturated, a decrease in [ATP] plays some role, whereas when the system is fully saturated, the stimulation is not transduced at all. At low work intensities, the total increase in V\textsubscript{O\textsubscript{2}} is slightly lower than at higher (but before saturation) work intensities because of a significant contribution (up to 60% in rest) of proton leak to V\textsubscript{O\textsubscript{2}} at low work intensities. Namely, an increase in relative energy demand decreases \( \Delta p \) and thus decreases proton leak, which leads to a partial compensation of the increase in V\textsubscript{O\textsubscript{2}} caused by an elevated energy demand.

On the other hand, in the each-step-activation mechanism, almost half of the V\textsubscript{O\textsubscript{2}} stimulation is transduced by direct parallel activation of particular oxidative phosphorylation steps, the contribution of an increase in [ADP] to the V\textsubscript{O\textsubscript{2}} stimulation is very similar to the contribution of direct activation, and the contribution of an increase in [Pi] is below 10%, whereas a decrease in [ATP] plays essentially no role. Therefore, in the each-step-activation mechanism, about half of the stimulation of V\textsubscript{O\textsubscript{2}} is due to the increase in [ADP] and [Pi], whereas the remaining half is due to the direct activation of oxidative phosphorylation.

Figure 5 presents the simulated pattern of metabolic control of particular components of the oxidative phosphorylation system over the oxygen consumption flux (A) and ATP turnover flux (B) at different energy demands (between 1 and 100) in the case of the output-activation mechanism. It can be seen
(Fig. 5A) that at rest (relative energy demand 1), the proton leak through the inner mitochondrial membrane has the highest control over $V_{O2}$ (flux control coefficient equal to 0.48); this is due to the fact that ~60% of oxygen consumption in skeletal muscle at rest is due to proton leak (45, 46). ATP usage keeps 35% of the control, and the remaining 17% is shared among particular oxidative phosphorylation complexes and substrate dehydrogenation. As the energy demand increases, most of the control (over 90%) is taken over by ATP usage. However, when oxidative phosphorylation becomes saturated at an energy demand of 30, the flux control coefficient of ATP usage over oxygen consumption falls to zero and the control becomes distributed more or less uniformly (flux control coefficients are of the same order of magnitude) between oxidative phosphorylation complexes and substrate dehydrogenation.

The distribution of control over ATP turnover (Fig. 5B) is rather similar to that over $V_{O2}$. The main difference is that near the resting state, essentially all the control over ATP production/consumption is kept by ATP usage, whereas proton leak, oxidative phosphorylation, and substrate dehydrogenation possess almost no control.

Figure 6 presents the simulated pattern of control over oxygen consumption (A) and ATP turnover (B) in the case of the each-step-activation mechanism. In the vicinity of the resting state, the distribution of control is essentially the same as for the output-activation mechanism. However, when the relative energy demand progressively increases up to 100, ATP usage continues to keep almost 100% of the control over both $V_{O2}$ and ATP production/consumption, whereas the control exerted by other steps is essentially 0%. Therefore, the control is not taken over by ATP-producing processes even at high values of energy demand.

Figure 7 presents the simulated dependence of $V_{O2}$ and metabolite concentrations on (extracellular) oxygen concentration at high relative energy demand equal to 100 (the rightmost point in Figs. 3–6) in the case of the each-step-activation mechanism. As shown, $V_{O2}$ is essentially insensitive to oxygen concentration over a very broad range and begins to fall when $[O_2]$ decreases below 3 μM. Under the simulated conditions, the half-saturating extracellular $[O_2]$ for the respiration rate is slightly greater than 1 μM. However, several metabolite concentrations, including [ADP], [Pi], [PCr], and [Cr] (and also NADH/NAD+, reduction level of cytochrome $c$, and $\Delta p$; not shown, see Ref. 25) start to change at much higher oxygen concentrations. These changes represent the response of the system to low $[O_2]$: they lead to an activation of particular components of the system that counteracts the inhibition of the system caused by a decrease in oxygen concentration. As a result, the respiration rate decreases much less than it would in the absence of changes in intermediate metabolite concentrations, and the phenomenological (apparent) half-saturation constant of oxidative phosphorylation for oxygen is much smaller than the mechanistic Michaelis-Menten constant of cytochrome oxidase for oxygen (see below) (25, 55). At $[O_2]$ equal to ~3 μM, the capacity of oxidative phosphorylation for ATP production becomes exhausted and a critical point (analogous to the critical point in energy demand at the output-activation mechanism) appears, below which [PCr] and [ATP] fall to zero, [Cr] and [Pi] rise to the maximal values, and [ADP] first rises and then decreases, being progressively converted to AMP by adenylate kinase (as a consequence, essentially all adenine nucleotides are in the form of AMP).

It is worthwhile to emphasize that the mechanistic (for constant $\Delta p$ and reduction level of cytochrome $c$; reduction level of cytochrome $a_3$ is a function of these variables) Michaelis-Menten constant of cytochrome oxidase for oxygen (compare Eq. 2) is much greater (120 μM) than the phenomenological half-saturation constant of oxidative phosphorylation for oxygen. The difference is due to the compensation of the decrease in oxygen concentration by an increase in the reduction level of cytochrome $c$ and decrease in $\Delta p$ (25, 28). It should be also stressed that the value of the apparent (phenomenological) half-saturation constant depends on the relative
The main aim of the present study was to analyze the impact of the each-step-activation mechanism of the regulation of oxidative phosphorylation during muscle contraction proposed previously (26–29) on the pattern of metabolic control over the oxygen consumption and ATP turnover flux exerted by ATP usage, substrate dehydrogenation, and particularly oxidative phosphorylation complexes at different energy demands in intact skeletal muscle. In particular, it was important to know how this mechanism influences the flux control coefficient of ATP usage at higher energy demands and, therefore, how much an increase in the activity of ATP usage at higher work intensities can further increase the V\(_{\text{O}_2}\) and ATP production/consumption flux. In addition, the question was addressed as to how evenly the metabolic control over the flux is distributed between particular oxidative phosphorylation complexes when ATP production by oxidative phosphorylation becomes saturated, and therefore how many complexes must be directly activated to cause a great increase in the flux. Finally, we analyzed how low oxygen concentration affects the pattern of metabolic control and therefore how it can determine the maximal respiration rate and ATP turnover in skeletal muscle.

First of all, computer simulations demonstrated (Fig. 3) that the each-step-activation mechanism helps to maintain much more constant metabolite concentrations in a much wider range of energy demand than the output-activation mechanism does. This is very important for the distribution of the metabolic control, because as long as ATP concentration is much higher than the \(K_m\) constant of ATP usage for ATP (equal to 150 \(\mu\)M within the model) and therefore ATP usage is essentially saturated with ATP, this process will keep most of the control (within Metabolic Control Theory, flux control coefficients are inversely proportional to elasticity coefficients quantifying the sensitivity of the reaction rate to metabolite concentrations). When [ATP] drops to values similar to or lower than the \(K_m\) constant of ATP usage for ATP, the rate of ATP usage becomes strongly dependent on [ATP] and other processes take control over the flux. Of course, [ATP] is related to [ADP], [AMP], [P], [PCr], [Cr], and other metabolite concentrations, and therefore a possibly high constancy of these concentrations in relation to low-work concentrations is crucial for maintaining a high value of the flux control coefficient of ATP usage. This in turn enables a further increase in the oxygen consumption and ATP turnover flux when the rate constant of ATP usage (energy demand) is increased.

Figure 3 demonstrates that the each-step-activation mechanism causes a much greater maximal increase in the flux than the output-activation mechanism and, at the same time, only moderate changes in [ADP]. As a result, the phenomenological V\(_{\text{O}_2}/\text{ADP}\) relationship is much steeper here, and the maximal
VO₂ (when recalculated for the amount of mitochondrial proteins) is much larger than in isolated mitochondria (8, 16, 19, 36, 52).

Comparison of Fig. 6B with Fig. 5B shows that in the case of the each-step-activation mechanism, ATP usage keeps almost 100% of control over ATP turnover in the whole range of energy demand, whereas in the case of the output-activation mechanism, the control is taken over by oxidative phosphorylation complexes and substrate dehydrogenation above the critical energy demand of ~30. The situation is similar for the control over the oxygen consumption flux (Figs. 5A and 6A), except that the proton leak exerts substantial control in the near vicinity of the resting state, as found in experimental studies by Rolfe and Brand (45). The critical energy demand is also the point where [ATP] falls very close to zero and other metabolite concentrations quickly change and reach their final (high energy demand) values.

When oxidative phosphorylation becomes saturated above the energy demand of 30 in the case of the output-activation mechanism, the control is shared more or less evenly (flux control coefficients are of the same order of magnitude) by particular oxidative phosphorylation complexes, and the flux cannot be further increased by elevating energy demand (rate constant of ATP usage) (Fig. 5). Figure 6 demonstrates that the flux can be easily further increased (above the critical energy demand for output activation) if all oxidative phosphorylation complexes and substrate dehydrogenation are directly activated by some cytosolic factor in parallel with the activation of ATP usage, and therefore the flux control coefficient for ATP usage continues to be close to unity. However, the question arises as to whether it is necessary to activate directly all oxidative phosphorylation complexes or whether it would be enough to activate only some of them, or even only the substrate dehydrogenation system. The answer to this question lies in the pattern of control in the “saturated state” (an equivalent of state 3 in isolated mitochondria). Namely, if most of the control were kept by a few (or maybe only one) step(s) of the ATP-producing system, it would suffice to activate directly just those few (or one) step(s). On the other hand, if, as in the case presented in Fig. 5, the control is distributed more or less uniformly among all steps (for no step, its flux control coefficient is very close to zero), all these steps must be directly activated to account for the increase in VO₂ and [ADP] during rest-to-work transition in skeletal muscle encountered in experimental studies (if even one step is not directly activated, the activation of other steps would increase its flux control coefficient to near 1). The model used in the present study was extensively tested for the values of flux control coefficients of particular oxidative phosphorylation complexes in state 3 in isolated skeletal muscle mitochondria (35). It has been demonstrated that only the activation of all steps (each-step activation) can account for the behavior of the system in intact skeletal muscle during rest-to-work transition (26). A similar situation prevails in intact heart in vivo, where only a well-balanced parallel activation of ATP usage, substrate dehydrogenation, and all oxidative phosphorylation complexes is able to explain (33) the perfect stability of [ADP], [Pi], PCr/ATP, and NADH/NAD⁺ during low-to-high work transition (2, 14, 21, 50). Fell and Thomas (10) demonstrated in a more abstract and general way (within the frame of their “multisite modulation” concept) the necessity of the direct activation of many steps to increase significantly the flux when the control is distributed. Generally, in some important sense, the idea of each-step activation or multisite modulation can be regarded as a direct consequence of the theoretical and experimental development of MCA (9, 15, 20).

Figures 7 and 8 show that at low extracellular oxygen concentration (<3 μM), [ATP] falls to zero and the control over the flux is shifted from ATP usage to oxidative phosphorylation (in particular, cytochrome oxidase) [such an effect of low oxygen concentration was first predicted theoretically (34) and then confirmed experimentally (54)]. The effect of low oxygen concentration is in a sense opposite to the effect of each-step activation (see Figs. 3A and 5).

It has been postulated that the intensity of the each-step-activation (parallel activation) is high in oxidative skeletal muscle under physiological conditions, moderate in glycolytic (but mitochondria rich, containing type IIA fibers) muscle under physiological conditions, and low or nonexistent in electrically stimulated perfused glycolytic muscle (29). The simulations performed in the present study concern mostly the first case, high each-step activation in oxidative skeletal muscle in vivo stimulated neurally (voluntary exercise). As shown in Fig. 3B, this mechanism offers a possibility of a very high increase in the oxygen consumption (and ATP turnover) flux up to very high values. In fact, computer simulations show that for a relative energy demand of 200, VO₂ increases to 24 mM/min (and [PCr] decreases to 3.4 mM), and for a relative energy demand of 250, VO₂ increases to 30 mM/min (and [PCr] decreases to 1 mM), whereas for the saturating (in the case of high each-step activation) relative energy demand equal to 370, VO₂ reaches 38 mM/min (and [PCr] drops to near zero). The maximal VO₂ in human skeletal muscle equals 16 mM/min in single muscle exercise (1, 3, 36, 48) [in one experiment, the value of 26 mM/min was obtained (44)] and is about twice as low in whole body exercise (e.g., cycling) (36, 48, 52). One possible explanation of this difference in humans can be a limitation of oxygen transport by blood (48), leading to a decrease in muscle oxygen concentration and thus to limitation of the respiration rate (compare Figs. 7 and 8). On the other hand, it has been postulated that in quadrupeds, this limitation of the cardiovascular system does not exist (47, 49). Therefore, the maximal VO₂ during single muscle exercise in humans and during whole body exercise in quadrupeds may be simply determined by the maximal rate of ATP usage during sustained exercise by muscles.

It also has been postulated that in quadrupeds, the whole animal maximal sustained oxygen uptake is similar, when recalculated for the mitochondria volume, to the maximal oxygen uptake in isolated mitochondria (17, 49). However, a detailed analysis of the presented experimental data demonstrates that this conclusion is not so straightforward. First, VO₂ per mitochondria volume during whole body spontaneous exercise in quadrupeds is almost twice that in isolated mitochondria incubated with the “physiological” substrate (product of glycolysis) pyruvate (17, 49) (succinate and, especially, cytochrome c are not physiological substrates of oxidative phosphorylation in skeletal muscle; with both substrates the maximal VO₂ is overestimated because succinate is an FAD-related substrate that “omits” complex I and the “key,” rate-controlling TCA cycle dehydrogenases, whereas reduced cytochrome c is a substrate of cytochrome oxidase, the activity of...
which is much greater than the activity of entire oxidative phosphorylation; under physiological conditions, cytochrome c is reduced 20–30% and not 100%). If it is taken into account that isolated mitochondria are incubated with saturating [ADP], [O₂], and respiratory substrate concentrations, one can estimate that the “true” capacity of oxidative phosphorylation in quadrupeds during whole body exercise is at least twice greater than in isolated mitochondria. Second, maximal VO₂ per mitochondria volume in quadrupeds is at least three times greater during spontaneous whole body exercise than during single muscle electrically stimulated exercise (Table 1 in Ref. 17). Both results agree well with our proposal that the presence of each-step activation results in much greater maximal VO₂ in neurally stimulated muscles than in electrically stimulated muscles or in isolated mitochondria.

The distribution of metabolic control over the ATP turnover flux between ATP supply and ATP usage at different energy demands simulated for the output-activation mechanism, presented in Fig. 5B, is similar to that calculated on the basis of experimental data and their extrapolation by Jeneson et al. (18). The authors demonstrated that in glycolytic skeletal muscle stimulated electrically, ATP usage (actomyosin-ATPase and Ca²⁺-ATPase) has almost entire control over ATP turnover at low (0.3–2 Hz) stimulation frequency; at moderate (3 Hz) stimulation frequency, half of the control is taken over by ATP supply that becomes an almost fully controlling step (flux control coefficient ~0.9) at high (10 Hz) stimulation frequency. In fact, the experimental data obtained covered the range of stimulation frequency between 0.3 and 1.8 Hz, whereas the flux control coefficients for higher frequencies were calculated using a simple kinetic model developed by the authors. It can be estimated on the basis of the experimental data presented in Tables 1–3 in Ref. 18 that ATP turnover increases ~20 times between rest (extrapolation to 0-Hz stimulation frequency) and the maximal stimulation frequency (1.8 Hz), which caused a sustained muscle work in the discussed experimental system (at higher frequencies, muscle work ceased quickly because of fatigue). This finding agrees well with the computer simulations presented in Fig. 3A if it is assumed that muscle becomes fatigued when the ATP/ADP ratio falls below ~50. Therefore, in real conditions, muscle ceases to work (because of fatigue) before ATP production takes control over the ATP turnover flux. Generally, it seems that, as has been discussed above and elsewhere (29), there is no each-step activation (parallel activation) in electrically stimulated glycolytic muscle.

Of course, the main criticism that may be addressed to the each-step-activation mechanism is that the physical nature of the factor/mechanism X that activates in parallel all oxidative phosphorylation complexes is somewhat unclear. From among the known factors, some mechanism involving calcium ions remains the most attractive candidate. Because high constant [Ca²⁺] only moderately activates isolated mitochondria (22, 43), it has been postulated (27) that the relevant factor may be the frequency of calcium oscillations. This frequency could be integrated over time by some protein, analogous to calmodulin, that is lost or inactivated during isolation of mitochondria. Recent studies have shown that calcium ions are tunneled from sarcoplasmic reticulum to mitochondria in skeletal muscle (51). Nevertheless, the oxidative phosphorylation complexes, being transmembrane proteins located in the inner mitochon-
present article concern only the rate of oxygen consumption and oxidative ATP production.

In summary, the present theoretical study demonstrates that the each-step-activation mechanism of the regulation of oxidative phosphorylation during muscle contraction has a great impact on the control exerted by particular elements of the ATP supply-demand system over the oxygen consumption and ATP turnover flux in skeletal muscle. Namely, this mechanism prevents the shifting of metabolic control from ATP usage to oxidative phosphorylation complexes when the energy demand (rate constant of ATP usage) increases. As a consequence, VO₂ and oxidative ATP production may potentially increase to very high values, even higher than those observed under physiological conditions in skeletal muscle. It is also shown that at low oxygen concentrations, the control over the flux is shifted from ATP usage to cytochrome oxidase, which prevents a further increase in VO₂ and ATP turnover caused by an increase in energy demand. Therefore, it is concluded that in skeletal muscle, especially in oxidative skeletal muscle where the each-step-activation is highest, it is not the potential capacity of oxidative phosphorylation but oxygen delivery by blood that determines the maximal oxygen consumption, oxidative ATP production, and sustained work intensity.

REFERENCES


