Regulation of ATP supply during muscle contraction: theoretical studies

Bernard KORZENIEWSKI

Institute of Molecular Biology, Jagiellonian University, al. Mickiewicza 3, 31-120 Kraków, Poland

The dynamic computer model of oxidative phosphorylation developed previously and successfully tested for large-scale changes in fluxes and metabolite concentrations was used to study the question of how the rate of ATP production by oxidative phosphorylation is adjusted to meet the energy demand during muscle contraction, which causes a great increase in ATP consumption in relation to the resting state. The changes in the respiration rate and ATP/ADP ratio after the onset of maximal work measured experimentally were compared with simulated changes in the respiration rate and ATP/ADP in several different cases, assuming direct activation of different steps by an external effector. On the basis of the computer simulations performed, it was possible to conclude which enzymes/metabolic blocks should be directly activated to cause the experimentally observable changes in fluxes and metabolite concentrations. The theoretical results obtained suggest that the parallel direct activation of actinomyosin-ATP-ase and oxidative phosphorylation by an external effector (for example calcium ions) is the main mechanism responsible for fitting of ATP production to ATP consumption, while the negative feedback via an increase in ADP concentration (decrease in ATP/ADP), which indirectly activates the ATP supply, plays only a minor role. Additionally, the conclusion is drawn that most of the oxidative phosphorylation steps should be directly activated in order to explain the observed changes in the respiration rate and ATP/ADP ratio (and also in other parameters) during muscle contraction. It is suggested that there should exist a universal external activator/regulatory mechanism which causes a parallel stimulation of different enzymes/processes. A possible nature of such an activator is shortly discussed.

INTRODUCTION

During the transition from the resting state of muscles to their maximal exercise, there is a great increase in energy demand (ATP consumption) [1–7]. Mitochondrial oxidative phosphorylation is the main process responsible for ATP production in most muscle fibre types under most conditions. Therefore, mitochondria have to 'know' in some way how fast should they produce ATP in a given moment of time to meet the rate of energy consumption and to avoid a drastic decrease in cytosolic phosphorylation potential which would hinder muscle contraction. When a muscle cell receives a neural signal of contraction, both the actinomyosin-ATP-ase and the ATP-producing reactions must be directly or indirectly activated. It is well known that Ca^{2+} is the intracellular activator of the ATP-utilizing block. On the other hand, the mechanisms underlying the stimulation of the ATP-producing block and the way in which the relevant regulatory signals are transferred remain a matter of debate.

Generally, two alternative mechanisms of the adjusting the energy (ATP) production rate to the energy consumption rate have been proposed. They can be called the 'negative feedback' and 'parallel activation' (Figure 1).

It is postulated within the framework of the negative feedback paradigm that only the ATP-consuming processes (mainly actinomyosin-ATP-ase and Ca^{2+} cycling) are directly activated by an external effector (for example calcium ions). The increase in the ATP usage during contraction causes a drop in ATP concentration as well as an increase in the concentration of ADP and inorganic phosphate. Because ATP inhibits, while ADP and P_i stimulate, respiration and ATP synthesis in mitochondria, these changes speed up the energy supply and equilibrate ATP production with ATP consumption, and therefore counteract a complete exhaustion of ATP. Different primary signals constituting this negative feedback have been proposed. Their list includes ADP concentration [8,9], the ATP/ADP ratio [10,11], ATP/$ADP \times P_i$ ratio [12], phosphorylation potential [13] and Atkinson's adenylate energy charge [14]. The original proposition by Chance and Williams probably remains the most popular idea, especially among physiologists dealing with skeletal muscle. It says that an increase in [ADP] is the main signal increasing ATP production. From the formal point of view, an increase in the ADP concentration is quantitatively equivalent to a decrease in the ATP/ADP ratio, since [ATP] is in muscle more than two orders of magnitude greater than [ADP] and the moiety-conservation property ([ATP] + [ADP] = const.) takes place (AMP concentration is negligible under most physiological conditions).

Due to the parallel activation paradigm, not only the ATP consumption, but also different steps inside the ATP production block are parallel and directly activated by an external effector, for example calcium ions. This paradigm was formulated for the first time when the stimulation of the irreversible tricarboxylate acid cycle dehydrogenases (pyruvate dehydrogenase, isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase) by calcium ions had been discovered [15–18]. Later reports, concerning stimulation by calcium ions also of the ATP synthase [19] and ATP/ADP carrier [20], reinforced the 'multi-site activation' hypothesis [21]. However, all these studies provided rather few data that would allow us to assess the quantitative significance of the parallel stimulation of different steps of the ATP production block.

Both paradigms, at least in principle, can be distinguished experimentally, because they give different quantitative predictions. Parallel activation implies much smaller changes in the ATP/ADP ratio after an activation of respiration (and ATP synthesis) flux than negative feedback does (see Figure 1). In the extreme case of perfectly equal activation of the ATP-consuming block and ATP-producing block, no changes in [ATP] and [ADP] should be observed.

* To whom correspondence should be addressed.

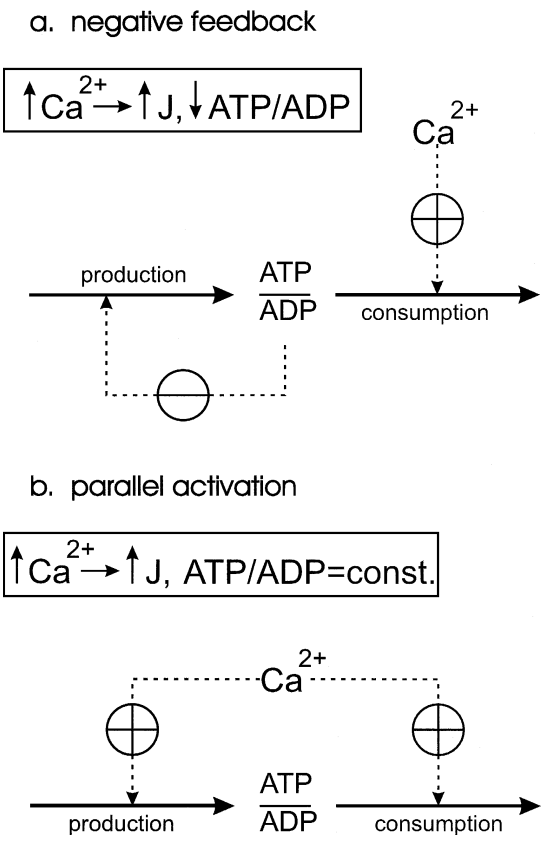

Figure 1 Comparison of the negative feedback and parallel activation mechanisms

(**a**) Negative feedback; only ATP consumption is activated directly by an external effector (for example calcium ions), while ATP production is activated indirectly, via a significant decrease in the ATP/ADP ratio. (**b**) Parallel activation; both ATP consumption and ATP production are directly activated by an external effector; the ATP/ADP ratio remains approximately constant.

The traditional idea of the negative feedback via ADP concentration was seriously shaken when Balaban and colleagues [22–25] published the results of their studies on the regulation of ATP production in the working heart *in vivo*. They reported that a five-fold increase in the respiration rate during increased work was accompanied by only 0–20 % increase in cytosolic ADP concentration. It is not possible to explain these results exclusively on the basis of the negative feedback concept. Many other studies [26–31] showed generally greater relative variations in ADP concentration during an increase of work intensity in heart. However, all these measurements were performed on perfused heart and therefore the conditions were not fully physiological. On the other hand, in heart perfused with glucose and insulin, [ADP] increased by only 6 % when the respiration rate increased 2.8 times [27]. This fact suggests an important role of hormones in balancing of ATP supply and ATP usage *in vivo* (hormones are absent in most perfusion mediums).

The situation is apparently not so clear in skeletal muscle. The concentration of ADP usually increases 3–5 times during contraction. Kushmerick et al. [32] found comparable relative changes in the respiration rate and ADP concentration, consistent with a simple Michaelis–Menten relationship and therefore with the negative feedback mechanism. However, these data covered only approx. 10 % of the true metabolic scope for activity of the cat muscles they studied, as discussed by Hochachka [1]. When the maximal power outputs and respiration rates are considered, relative changes in the oxygen consumption are much greater than changes in [ADP]. Some examples are given in Table 1. The most striking case is insect flight muscle, where respiration increases 600 times, while [ADP] grows only two times, and greyhound biceps femoris, where a 200-fold ATP turnover increase is accompanied by an only 4–5-fold increase in the ADP concentration. Even in a very 'moderate' case of dog gastrocnemius, transition from the resting state to maximal work is related to an 18-fold increase in oxygen consumption, while [ADP] experiences only an up-to-3-fold increase. All these examples were discussed by Hochachka [1] as evidence against the simple Michaelis–Menten interpretation of the relationship between $\dot{V}O_2$ and [ADP], based on the negative feedback mechanism.

Table 1 Changes in [ADP] and oxygen consumption during maximal muscle exercise in different organisms and muscle types

Organism/muscle	Increase in $\dot{V}O_2$ (times)	Increase in [ADP] (times)	Source
Rat gastrocnemius	10	2–2.5	[1,2]
Dog gastrocnemius	18	2–3	[1,3,4]
Greyhound biceps femoris	200	4–5	[1,5]
Thoroughbred leg muscle	60	~ 2	[1,6]
Human calf muscle	15	5	[1]
Insect flight muscle	600	2	[1,7]

Generally, one can pose two main questions concerning the regulation of the adjustment of ATP production to ATP consumption in contracting muscle: (1) Which mechanism predominates in equilibration of energy demand and supply: negative feedback or parallel activation? If the latter, it means that the ATP-producing block is directly activated by an external effector to a considerable extent. (2) Which steps in the ATP-producing block are directly activated by the external effector and to what extent?

In the present article the above problems are studied by means of the computer model of oxidative phosphorylation in skeletal muscle mitochondria developed previously [33]. The theoretical results obtained allow us to assess in a quantitative manner which components of oxidative phosphorylation should be activated, and to what extent, to obtain the changes in the respiration rate and [ADP] (ATP/ADP ratio) observed experimentally after an onset of maximal skeletal muscle work.

MODEL

Theoretical studies in this paper are based on the previously developed model of oxidative phosphorylation in isolated rat skeletal muscle mitochondria respiring on pyruvate [33,34]. This model yielded a correct value of the respiratory control ratio (respiration in state 3 to respiration in state 4), equal to about 9–10 [35], and therefore reflected properly the overall capacity of mitochondria for ATP synthesis, when this process was stimulated by a decrease in the ATP/ADP ratio. Moreover, the model imitated the inhibitor titration curves for particular components of oxidative phosphorylation very well [33], proving that it is suited for studies of the effect of variations in activities of different enzymes on changes in fluxes and metabolite concentrations. The model can be used in particular to state which steps should be directly activated, and to what extent, to yield the changes in the respiration rate as well as in the ATP/ADP ratio

Table 2 Kinetic descriptions of the components of oxidative phosphorylation taken into account explicitly in the model

Chosen parameters and variables: K_{mN}, Michaelis–Menten constant of substrate dehydrogenation for the $NAD^+/NADH$ ratio; K_{mO}, Michaelis–Menten constant of cytochrome oxidase for oxygen at constant Δp and reduction level of cytochrome c (apparent constant, at varying Δp and c^{2+}/c^{3+}, is much lower); K_{mA}, Michaelis–Menten constant of ATP usage for ATP; K_{mADP}, Michaelis–Menten constant of the ATP/ADP carrier for free external ADP; K_{mC1}, K_{mC3}, 'Half saturation' constants of complexes I and III for their thermodynamic spans; ΔE_{C1}, ΔE_{C3}, Thermodynamic spans of complexes I and III; ΔG_{SN}, ΔG_{EX}, Thermodynamic spans of reactions catalysed by ATP synthase and ATP/ADP carrier; a^{2+}, c^{2+}, Concentrations of the reduced form of cytochromes a_3 and c; p_D, Relative 'sensitivity coefficient' of substrate dehydrogenation to the $NAD^+/NADH$ ratio; k_{LK1}, k_{LK2}, Constants used in a phenomenological description of proton leak; k_{fAK}, k_{bAK}, Forward and backward rate constants of adenylate kinase. Subscripts: e, external; i, internal; t, total; f, free; m, magnesium complex.

Substrate dehydrogenation

$$v_{DH} = k_{DH}\frac{1}{\left(1+\frac{K_{mN}}{NAD^+/NADH}\right)^{p_D}} \qquad K_{mN} = 100, \quad p_D = 0.6$$

Complex I

$$v_{C1} = k_{C1}\frac{1}{1+(K_{mC1}/\Delta E_{C1})}, \qquad K_{mC1} = 2.5\ \text{mV}$$

Complex III

$$v_{C3} = k_{C3}\frac{1}{1+(K_{mC3}/\Delta E_{C3})}, \qquad K_{mC3} = 2.1\ \text{mV}$$

Complex IV

$$v_{C4} = k_{C4}\, a^{2+}\, c^{2+}\frac{1}{1+(K_{mO}/O_2)}, \qquad K_{mO} = 120\ \mu\text{M}\ (K_{mO}^{\text{apparent}} = 0.8\ \mu\text{M})$$

ATP synthase

$$v_{SN} = k_{SN}\left(\frac{\gamma-1}{\gamma+1}\right), \qquad \gamma = 10^{\Delta G_{SN}\cdot F/Z}$$

ATP/ADP carrier

$$v_{EX} = k_{EX}\left(\frac{ADP_{fe}}{ADP_{fe}+ATP_{fe}\,10^{-\Psi_e/Z}}-\frac{ADP_{fi}}{ADP_{fi}+ATP_{fi}\,10^{-\Psi_i/Z}}\right)\times\left(\frac{1}{1+K_{mADP}/ADP_{fe}}\right), \qquad K_{mADP} = 3.5\ \mu\text{M},$$

Phosphate carrier

$$v_{PI} = k_{PI}\,(Pi_e\,H_e - Pi_i\,H_i),$$

ATP usage

$$v_{UT} = k_{UT}\frac{1}{1+(K_{mA}/ATP_{te})}, \qquad K_{mA} = 15\ \mu\text{M},$$

Table 3 The set of differential equations, expressing the rates of metabolite concentration changes in time (the volumes of internal and external compartments as well as metabolite buffering pools are not indicated for simplicity)

$u = \Delta\Psi/\Delta p$; $n_A = 2.5$, H^+/ATP stoichiometry.

$$\dot{NADH} = v_{DH} - v_{C1}$$
$$\dot{UQH}_2 = v_{C1} - v_{C3}$$
$$\dot{c}^{2+} = v_{C3} - v_{C4}$$
$$\dot{O}_2 = 0 \quad \text{(constant saturated oxygen concentration)}$$
$$\dot{H}_i^+ = (2+2\,u)\,v_{C4} + (4-2\,u)\,v_{C3} + 4\,v_{C1} - n_A\,v_{SN} - u\,v_{EX} - (1-u)\,v_{PI} - v_{LK}$$
$$\dot{ATP}_{ti} = v_{SN} - v_{EX}$$
$$\dot{Pi}_{ti} = v_{PI} - v_{SN}$$
$$\dot{ATP}_{te} = v_{EX} - v_{UT} + v_{AK}$$
$$\dot{ADP}_{te} = v_{UT} - v_{EX} - 2\,v_{AK}$$
$$\dot{Pi}_{te} = v_{UT} - v_{PI}$$

(and also in other metabolite concentrations) observed experimentally. Studies of this kind are discussed in the present article.

Additionally, the model was successfully used in studies on the genesis of myopathies (caused by inborn mitochondrial enzyme deficiencies) and their tissue specificity as well as on the control of oxidative phosphorylation at low oxygen concentrations [33,34].

The model takes into account explicitly the following components of oxidative phosphorylation: substrate dehydrogenation (NADH supply), respiratory chain (complex I, complex III and complex IV), proton leak, ATP synthase, ATP/ADP carrier, phosphate carrier, ATP utilization and adenylate kinase. The kinetics of each step are described by an appropriate kinetic equation, expressing the dependence of the rate of this step on different metabolite concentrations. The Michaelis–Menten kinetics with ATP as the substrate were used for the ATP usage since the exact kinetics of the ATP utilization in muscle cells, especially of the actinomyosin-ATP-ase, are not known. Generally, a low value of the Michaelis–Menten constant (high affinity for ATP) was assumed [1]. The theoretical results obtained in this paper were similar for values of the Michaelis–Menten constant varying throughout five orders of magnitude, ranging from 1.5 nM to 1500 μM. The value of the Michaelis–Menten constant of the ATP usage for ATP equal to 15 μM was used in simulations as representative for this range. Other types of kinetics tested, with a greater sensitivity to adenylate nucleotide concentrations, for example a linear dependence on the ATP/ADP ratio, even strengthened the necessity of parallel activation, concluded for the Michaelis–Menten kinetics with ATP as the substrate. Therefore, the conclusions drawn in the present paper are essentially independent of the kinetics of the ATP usage. The kinetic equations for particular steps are presented in Table 2.

The rates of changes of different metabolite concentrations, constituting independent variables, in time were expressed as a set of ordinary differential equations. Such a set is presented in Table 3. This set was integrated numerically, by means of a computer. The Gear integration procedure was used and the simulation programs were written in Microsoft FORTRAN programming language.

It was assumed that the respiration in muscle in the resting state is 2 times greater than in state 4 in isolated mitochondria, on the basis of the observation made by Rolfe and Brand [36] that proton leak accounts for about 50 % of oxygen consumption in resting muscle. In this state the calculated cytosolic ATP/ADP ratio was equal to 258. The resting state was accepted as the reference state for further simulations and the respiration rate in this state was set up as equal to 1 (in arbitrary units). The simulations consisted of an activation of one or more enzymes (processes), which caused a transition of the system to a new ('active') steady state with a new respiration rate and ATP/ADP ratio, which were recorded. An activation of a given step n times was equivalent the n-fold increase in the rate constant or maximal velocity of this step in relation to the resting-state value.

Of course, the model of oxidative phosphorylation in isolated muscle mitochondria lacks some elements of the energetic system present in intact muscle, chiefly the creatine kinase system. However, the PCr/Cr pair does not change the kinetic response of mitochondria *in vivo* to the ATP/ADP ratio, serving only as an energy-equivalent buffering pool and diffusion facilitation system [37]. Some reports suggested ADP diffusion limitations in artificial isolated mitochondria [38] and skinned fibre [39] systems lacking creatine kinase, Cr and PCr. However, since the model

does not assume any ADP diffusion limitations (thus certainly reflecting better the physiological intact muscle conditions than isolated mitochondria conditions) the CK system can be omitted without any consequences for the results obtained and conclusions drawn. Any significant ADP diffusion limitations (or significant displacement of the creatine kinase from equilibrium) would result in great changes in ADP concentration after an onset of contraction (much greater concentration gradients at high fluxes), which is not observed experimentally. A significant displacement of the creatine kinase from equilibrium would require also a direct activation of this step by an external effector during muscle contraction. As to the buffering of the ATP/ADP ratio by the PCr/Cr pair, this phenomenon can only change transition times between different steady-states, but not final fluxes and metabolite concentrations achieved in these states, which is what we are interested in the present article.

The simulations performed in the present paper represent well-oxygenated muscle with no significant pO_2 gradients. Low oxygen concentrations and/or oxygen diffusion limitations would even strengthen the need of parallel activation, as it is discussed below.

THEORETICAL RESULTS AND DISCUSSION

For the purposes of the present paper the rather moderate second case from Table 1 (dog gastrocnemius) is taken as representative for maximal muscle contraction. Therefore, it is assumed that in skeletal muscle during transition from the resting state to maximal work the respiration rate increases 18 times, which is accompanied by a 3-fold increase in the ADP concentration (decrease in the ATP/ADP ratio). The question to be answered by means of the computer model of oxidative phosphorylation is: which steps of this system should be directly activated by an external effector, and to what extent, to obtain the reported changes in the respiration rate and ATP/ADP ratio?

Of course, first the negative feedback hypothesis was checked, according to which the only step activated directly by an external signal is the actinomyosin-APT-ase (more generally: ATP usage), while the ATP-supplying reactions are stimulated indirectly, through changes in the ATP/ADP ratio (and in other metabolite concentrations, for example NADH/NAD^+, cyt c^{2+}/cyt c^{3+} ratio or Δp). The simulated changes in the respiration rate and the ATP/ADP ratio in the case when only ATP utilisation is activated are presented in Table 4. The first two rows present the values of these parameters in the resting state and the expected values in the active state (the respiration rate in the resting state is normalized to be equal to unity, in arbitrary units). The third row shows the simulated respiration rate and ATP/ADP ratio for two different degrees of the ATP usage activation. The expected decrease in the ATP/ADP ratio (three times) is achieved when the ATP consumption is activated about 3.3 times. However, such an activation causes a much smaller increase in the respiration rate than expected. On the other hand, when a very high activation of the ATP usage is imposed, the ATP/ADP ratio falls drastically while the respiration rate achieves a level still far below the experimentally-stated value. The results lead directly to the conclusion that the negative feedback mechanism cannot account for the 18-fold increase in the respiration rate and explains the only 3-fold changes in the ATP/ADP ratio.

Table 4 Simulated changes in the respiration rate and the ATP/ADP ratio when only the ATP utilization (UT) is activated

'X × *n*' means that step X is activated *n* times.

State	Respiration rate	ATP/ADP
Resting state	1	258
Expected active state	18 (↑ 18 ×)	86 (↓ 3 ×)
Simulated active state		
UT × 3.3	2.3	89
UT × 100	4.5	0.1

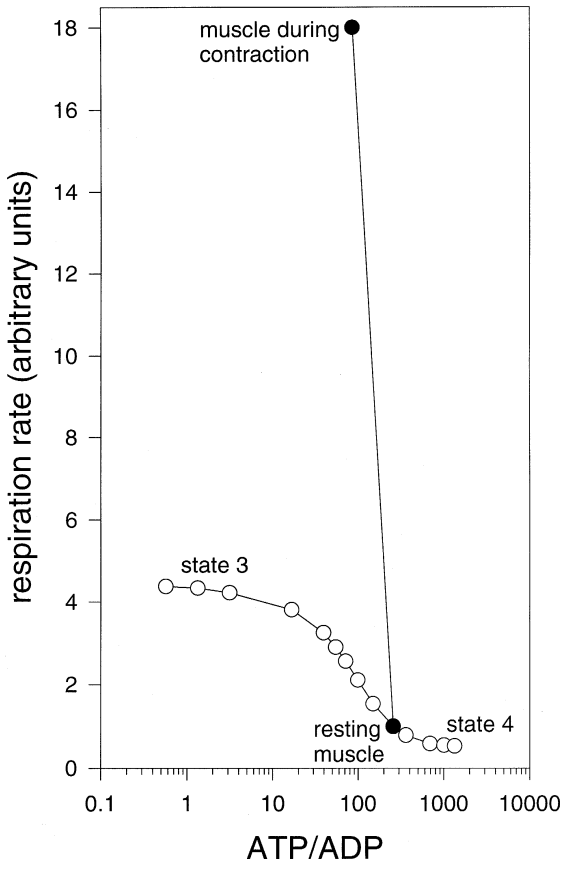

Figure 2 Comparison of the simulated kinetic response of muscle mitochondria to the ATP/ADP ratio (empty circles) with the increase in the respiration rate (18-fold) and decrease in the ATP/ADP ratio (three-fold) during transition from resting state to maximal work in skeletal muscle (dog gastrocnemius *in vivo* measured experimentally [1,3,4]) (full circles)

In fact, this conclusion is intuitively obvious. The respiratory control ratio of muscle mitochondria, equal to about 10, fixes the maximal capacity of oxidative phosphorylation for an increase in oxygen consumption (and ATP supply). Additionally, the respiration in resting muscle is about two times greater than in state 4 in isolated mitochondria [36], which, taken together, leaves the possibility for only an about 5-fold increase in the respiration rate in response to a decrease in the ATP/ADP ratio (see the last row in Table 4). This problem is illustrated in Figure 2, where a simulated kinetic response of muscle mitochondria to a decrease in the ATP/ADP ratio (similar to the sigmoidal dependence measured experimentally by Wanders and Westerhoff [40]) is compared with the changes in the respiration rate and the ATP/ADP ratio after an onset of maximal work. If the negative feedback is the only relevant mechanism, these two lines should overlap. Evidently, this is not the case. The increase in the respiration rate during muscle contraction is much greater than

Table 5 Simulated changes in the respiration rate and the ATP/ADP ratio when only the ATP utilization (UT) and substrate dehydrogenation (DH) are activated

'X × *n*' means that step X is activated *n* times.

State	Respiration rate	ATP/ADP
Resting state	1	258
Expected active state	18 (↑ 18 ×)	86 (↓ 3 ×)
Simulated active state		
UT × 3, DH × 50	3.0	88
UT × 20, DH × 50	5.2	0.4

Table 6 Simulated changes in the respiration rate and the ATP/ADP ratio when both the ATP utilization (UT) and the ATP production (all steps of oxidative phosphorylation besides the proton leak) are activated in parallel

LK, proton leak; DH, substrate dehydrogenation; C1, complex I; C3, complex III; C4, cytochrome oxidase; SN, ATP synthase; EX, ATP/ADP carrier, PI, phosphate carrier, 'X × *n*' means that step X is activated *n* times.

State	Respiration rate	ATP/ADP
Resting state	1	258
Expected active state	18 (↑ 18 ×)	86 (↓ 3 ×)
Simulated active state		
UT × 29, LK × 1, (DH, C1, C3, C4, SN, EX, PI) × 8.5	17.8	88

the capacity of mitochondria and the slope of the curve is much steeper for intact muscle than for isolated mitochondria. The latter property strongly suggests the parallel activation mechanism (compare Figure 1), where both ATP-consumption and ATP-production are parallelly activated and the ATP/ADP ratio changes relatively little. The relationship between the relative changes in flux J and intermediate metabolite M concentration after activation, on the one hand, and the kinetic responses of the M-producing and M-consuming block to M concentration, on the other hand, was discussed in greater detail in the frame of the proportional activation approach [41].

A certain increase in the reduction level of NAD (the NADH/NAD^+ ratio) during a work increase in heart prompted some authors to formulate the hypothesis that NADH supply is one of the main mechanisms regulating the respiration rate and ATP synthesis via an increase in NADH concentration [28]. To check this supposition, the effect of the parallel stimulation of the NADH supply (substrate dehydrogenation process, for example citric acid cycle dehydrogenases) and the ATP utilization (without activation of other steps) on the relative changes in the respiration rate and the ATP/ADP ratio was simulated. The theoretical results obtained are presented in Table 5. Even for a very high stimulation of the substrate dehydrogenation block (50 times) it was not possible to obtain the 18-fold increase in the respiration rate. For the parallel activation (together with NADH supply) of the ATP usage giving the desired decrease in the ATP/ADP ratio, namely 4.2-fold activation, respiration increased only three times. A strong activation of both the ATP usage and NAD supply resulted in a drastic drop in the ATP/ADP ratio (to below 1) and still very modest increase in the respiration rate (about five times). Additionally, in both cases the NADH/NAD^+ ratio experienced a very high increase, much greater than in the reported experiments [28,42]: NAD became almost completely reduced (reduction level near 100 %; not shown). Taken together, the above calculations suggest that during muscle contraction the NADH-producing processes are activated only slightly stronger than the NADH-consuming processes (which causes a small increase in the NADH/NAD^+ ratio) and that the parallel activation of only NADH supply and ATP usage cannot account for the observed changes in the respiration rate and the ATP/ADP ratio. This is because other steps become saturated far before the respiration rate reaches the level observed in muscle during maximal work.

Of course, small variations in intermediate metabolite concentrations (ATP/ADP, NADH/NAD^+ or Δp) as well as any desired increase in the respiration rate can be easily obtained when all components (enzymes, processes) of the considered system are activated to an appropriate extent. The 18-fold increase in the respiration rate and 3-fold decrease in the ATP/ADP ratio was obtained in computer simulations when ATP usage was activated 29 times while all the steps of oxidative phosphorylation (besides proton leak) were activated 8.5 times: see Table 6. (It was assumed that proton leak was not activated since this process does not participate in ATP synthesis and its activation would be equivalent to a net energy waste.) The 29-fold activation of ATP usage and 8.5-fold activation of each oxidative phosphorylation step is the unique solution giving the desired increase in the respiration rate and decrease in the ATP/ADP ratio, if it is assumed that each oxidative phosphorylation enzyme is activated to the same extent. However, a similar result can be obtained in the case where some enzymes of oxidative phosphorylation are activated slightly less, while other enzymes are activated slightly more. Nevertheless, the difference in the degree of activation cannot be great in order to avoid large changes in the cytosolic ATP/ADP ratio as well as in other parameters (NADH/NAD^+ ratio, UQH_2/UQ ratio, cytochrome *c* reduction level, Δp, mitochondrial ATP/ADP ratio)

The above theoretical result suggests that the experimentally observed relationship between relative changes in fluxes and metabolite concentrations can be, at least in principle, explained by parallel activation of ATP consumption and all the steps leading to ATP production. Different steps of the ATP-producing block should be activated to a comparable extent, but not necessarily to the same extent. Generally speaking, the parallel activation paradigm, understood as parallel activation of all the steps (and not only of the ATP-consuming block and ATP-producing block as a whole) offers a reliable explanation of the phenomena observed experimentally in contracting muscle.

However, the simulations discussed above show only that some, but not necessarily all, the components of mitochondrial oxidative phosphorylation have to be directly activated. The theoretical data indicate that activation of only ATP usage and NADH supply is not enough, while stimulation of all the steps suffices. It is still not clear whether an activation of only a part of the rest of the enzymes (together with the activation of the ATP usage and NADH supply) could yield the desired effect and, if so, which enzymes must be activated and which enzymes have a capacity great enough to enable the expected increase in the flux to occur without an external activation. To solve this problem, a set of computer simulations was performed where the degrees of activation of particular steps were identical as in the simulation presented in Table 6, but in subsequent simulations subsequent single steps were not activated. In other words, this time all the steps but one (different in each simulation) were activated (bear in mind that proton leak also was not activated for the reasons mentioned before). The influence of such a procedure on the changes in the respiration rate as well as in the

ATP/ADP ratio during activation is presented in Table 7. '−X' means that all the steps but step X were activated to the extent indicated in Table 6. The lack of an activation of a particular step for most steps results generally in a similar effect: the ATP/ADP ratio decreases drastically (below 1) while more or less half of the expected increase in the respiration rate is observed. The only exception is cytochrome oxidase which seems to have a capacity high enough to allow the system to achieve the desired increase in the respiration rate without a direct activation of this enzyme.

This conclusion is in agreement with the distribution of the metabolic control in skeletal muscle mitochondria in state 3, where oxidative phosphorylation is saturated and a further decrease in the ATP/ADP ratio does not cause any increase in the respiration flux. In muscle mitochondria (unlike in liver mitochondria) the control is shared more or less uniformly between different enzymes in state 3: essentially all the steps of oxidative phosphorylation (besides proton leak and cytochrome oxidase) have considerable (equal to or more than 0.1) values of flux control coefficients [35]. This means that all these steps become saturated approximately simultaneously when the flux through the system (stimulated by a decrease in the ATP/ADP ratio) increases. For this reason, all these steps have to be directly activated by an external effector to allow the increase in the respiration rate during muscle contraction observed experimentally, and to prevent a significant drop in the ATP/ADP ratio.

Summing up, to cause the 18-fold increase in the respiration rate and the 3-fold decrease in the ATP/ADP ratio in skeletal muscle during transition from the resting state to maximal work: (1) Both ATP consumption and ATP production should be directly activated; (2) ATP usage should be activated about 30 times and ATP supply about nine times; (3) most of the components of the ATP-producing block should be activated to a comparable extent.

Generally, the theoretical results obtained with the aid of the computer model of oxidative phosphorylation strongly suggest that the parallel activation is the main mechanism responsible for the increase in the respiration rate and ATP synthesis rate, while the negative feedback via ADP concentration constitutes only a secondary, 'fine tuning' mechanism. One can easily conclude that parallel activation of oxidative phosphorylation is responsible for about an 8-fold increase in the respiration rate, while the 3-fold decrease in the ATP/ADP ratio results in the remaining 2.2-fold increase of oxygen consumption ($8 \times 2.2 = 18$).

On the other hand, Kushmerick and colleagues [32] observed a more or less Michaelis–Menten relationship between the respiration rate and ADP concentration at very small stimulation of muscle contraction, with the ATP turnover equal to up to 10 % of the ATP turnover at the maximal work (compare discussion by Hochachka [1]). These results suggest that the negative feedback via [ADP] may indeed be the main mechanism responsible for the adjustment of the ATP supply rate to the ATP consumption at very low work intensities, while at medium and high work intensities the parallel activation mechanism is recruited and begins to predominate.

It must be stressed that the discrepancy between the experimental data concerning maximal muscle work intensity and the theoretical results obtained in simulations in which the lack of stimulation of the ATP-producing block, or even of single steps in this block, was assumed is so great (much lower respiration rate and the ATP/ADP ratio lower by several orders of magnitude) that it is extremely improbable that this discrepancy is a result of an inaccuracy in the model. The conclusions drawn would be valid even if the model reflected the properties of muscle oxidative phosphorylation only in a semi-quantitative way. However, the model seems to imitate very well the large-scale changes in fluxes and metabolite concentrations caused by changes in enzyme activities [33]. The model was able to mimic the inhibitor titration curves for particular components of oxidative phosphorylation and thus to reflect correctly the changes in the respiration rate and metabolite concentrations while activity of different enzymes gradually dropped from its normal value to zero [33]. The positive result of such a test is extremely important, because it justifies the use of the model for studies of the muscle contraction phenomenon, where large-scale changes in fluxes and metabolite concentrations occur as well. For all these reasons, the general conclusions formulated on the basis of the computer simulations discussed above seem to be inevitable.

It should be also taken into account that a rather moderate increase in the respiration rate during resting state → maximal work transition was taken as a reference for computer simulations (compare Table 1). The need for the parallel activation is even stronger in the case of the thoroughbred leg muscle, greyhound biceps femoris and, particularly, insect flight muscle. Additionally, the simulations presented in this article were performed for a high (saturated) oxygen concentration. It was shown in the earlier theoretical studies [34] that a decrease in the oxygen concentration to a few micromolar (very likely to occur in muscle during prolonged intensive work) essentially lowers the capacity of oxidative phosphorylation for the increase in the respiration rate (and ATP synthesis) caused by a decrease in the ATP/ADP ratio. This fact further increases the significance of the parallel activation as the mechanism ensuring an appropriate increase in the ATP synthesis rate due to the current energy demand in muscle.

The observation of Balaban and co-workers than in heart *in vivo* a 5-fold increase in the respiration rate is accompanied by an increase in [ADP] by only 0–20 % [22–25] strongly suggests that the parallel activation is an important regulatory mechanism in working heart. Additionally, theoretical studies performed with the aid of the computer model of oxidative phosphorylation in liver cells (hepatocytes) developed previously indicate that also Ca^{2+}-acting hormones (catecholamines), such as vasopressin, adrenaline and glucagon, stimulate parallelly the Δp-producing subsystem and the Δp-consuming subsystem in hepatocytes [43]. This theoretical prediction was next confirmed experimentally [41]. The relative activation of both subsystems, calculated in the frame of the proportional activation approach [41], were almost equal. The calculations performed for skeletal muscle gave a much better balance of the activation of the ATP-producing block and ATP-consuming block than that obtained in the present paper. However, the proportional activation approach can be applied quantitatively only to small (preferably infinitesimal) changes in fluxes and metabolite concentrations, and not to very large changes observed in skeletal muscle. Fell and Thomas [44,45] give many other examples where parallel activation seems to be the only reasonable explanation for the observed changes in fluxes and metabolite concentrations.

All these data, together with the theoretical results obtained in the present paper, suggest that the parallel activation of different enzymes by an external effector is a universal and widespread mechanism. Its function is probably to enable an appropriate increase in flux (especially in skeletal muscle) and to keep the concentrations of intermediate metabolites (such as ATP, NADH and acetyl-CoA) as constant as possible. These metabolites are substrates for numerous reactions, necessary for the functioning of the cell and keeping it alive. A significant decrease in their concentrations would seriously disturb such reactions.

Of course, the dynamic model of oxidative phosphorylation cannot answer the question concerning the nature of the universal

Table 7 Simulated changes in the respiration rate and the ATP/ADP ratio when the ATP utilization (UT) and all the steps of the ATP production but one (and proton leak) are activated in parallel

The step X which is not activated in a given case is marked as '—X'. LK, proton leak; DH, substrate dehydrogenation; C1, complex I; C3, complex III; C4, cytochrome oxidase; SN, ATP synthase; EX, ATP/ADP carrier, PI, phosphate carrier.

State	Respiration rate	ATP/ADP
Resting state	1	258
Expected active state	18 (↑18×)	86 (↓3×)
Simulated active state		
—DH	7.0	0.4
—C1	6.1	0.3
—C3	5.5	0.3
—C4	17.7	57
—SN	8.3	0.4
—EX	12.3	0.7
—PI	10.1	0.5

regulator and mechanistic aspects of parallel activation. However, the theoretical results obtained with the aid of the model clearly show the need for the identification of a general factor activating (almost) all the enzymes (carriers, processes) participating in oxidative phosphorylation to a comparable extent. It is not clear if the main known candidate for such an activator, namely calcium ions, exhibits all the necessary properties. The calcium ions hypothesis is not fully satisfactory, since their effect on many steps (citric acid cycle dehydrogenases, ATP synthase, ATP/ADP carrier) *in vitro* is too small and/or dubious to explain the phenomena occurring *in vivo* [15–20].

In this situation, three possibilities concerning the nature of the universal activator may be proposed: (1) Calcium ions are the factor sought. Their action is, for some reasons, more effective *in vivo* than *in vitro*; (2) calcium ions act *in vivo* via some protein, functionally analogous to calmodulin, which is lost or inactivated during mitochondria preparation; (3) in intact cells, another activator/mechanism (for example protein phosphorylation, latent enzymes recruitment [1] or changes in the mitochondrial matrix volume [17]) of yet unknown nature exerts an effect parallel to the direct effect of calcium ions on different enzyme activities.

The second possibility can be supported by the fact that the isolated ATP/ADP carrier is activated by calcium ions, but the concentration of calcium needed is at least two orders of magnitude greater than in intact cells [46]. This suggests that something mediates in 'presenting' calcium ions to the ATP/ADP carrier.

Summing up, the computer model of oxidative phosphorylation in skeletal muscle mitochondria shows that the only quantitatively valid explanation of the existing experimental data is that the parallel activation of different steps constitutes the main mechanism responsible for the adjustment of the ATP production rate to the current energy demand in working muscle and that the intuitive interpretations based on the negative feedback mechanism, although qualitatively logical, do not work when quantitative changes in fluxes and metabolite concentrations as well as the kinetic properties of mitochondria are taken into account.

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