ELSEVIER

Biophysical Chemistry 83 (2000) 19–34

Biophysical Chemistry

www.elsevier.nl/locate/bpc

Regulation of ATP supply in mammalian skeletal muscle during resting state → intensive work transition

Bernard Korzeniewski*

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

Received 26 June 1999; accepted 21 September 1999

Abstract

In the present debating paper, the problem how the rate of ATP supply by oxidative phosphorylation in mitochondria is adjusted to meet a greatly increased demand for ATP during intensive exercise of skeletal muscle is discussed. Different experimental results are collected from different positions of the literature and confronted with five conceptual models of the regulation of the oxidative phosphorylation system. The previously performed computer simulations using a dynamic model of oxidative phosphorylation are also discussed in this context. The possible regulatory mechanisms considered in the present article are: (A) output activation: an external effector activates directly only the output of the system (ATP turnover); (B) input/output activation: an external effector activates directly the output (ATP usage) and input (substrate dehydrogenation) of the system; (C) removal of substrate shortage: only ATP consumption and substrate supply by blood are directly activated; (D) removal of oxygen shortage: only ATP consumption and oxygen supply by blood are directly activated; (E) each step activation: an external effector activates both the ATP-consuming subsystem and all the steps in the ATP-producing subsystem (particular enzymes/carriers/blocks of oxidative phosphorylation, substrate supply, oxygen supply). The performed confrontation of the considered mechanisms with the presented results leads to the conclusion that only the each step activation model is quantitatively consistent with the whole set of experimental data discussed. It is therefore postulated that a universal effector/regulatory mechanism of a still unknown nature which activates all steps of oxidative phosphorylation should exist and be discovered. A possible nature of such an effector is shortly discussed.

Keywords: Oxidative phosphorylation; Skeletal muscle; Regulation of metabolism; Computer model

* Tel.: +48-12-634-13-05; fax: +48-12-633-69-07.
E-mail address: benio@mol.uj.edu.pl (B. Korzeniewski)

0301-4622/00/$ - see front matter
PII: S0301-4622(99)00120-9

1. Introduction

Oxidative phosphorylation is the main process producing ATP in most mammalian tissues under most conditions. Its general scheme, which takes into account the most important steps considered in the present paper, is presented in Fig. 1. Generally, the system comprises: ATP usage, different steps of oxidative phosphorylation in mitochondria (complex I, complex III, complex IV, proton leak, ATP synthase, ATP/ADP carrier, phosphate carrier), substrate dehydrogenation (system donating electrons on NAD) as well as substrate delivery and oxygen delivery by blood. The demand for ATP changes over time, depending on conditions. Therefore, when a cell receives a signal that some metabolic processes (driven by hydrolysis of ATP) within it are to be turned on or accelerated, the system of reactions which produce ATP must be also, directly or indirectly, activated in order to meet the increased rate of ATP consumption. The extracellular signals which stimulate metabolism (and therefore ATP turnover and respiration) in non-excitable tissues are mostly different hormones, while excitable tissues are stimulated by neural signals. The tissue where the greatest changes in the ATP turnover rate and respiration rate between resting state and maximal activation take place is skeletal muscle. A neural stimulation of skeletal muscle leads to a large activation of two ATP-consuming steps: actinomyosin-ATPase and Ca^{2+}-ATPase. Calcium ions are the secondary intracellular messenger, which is responsible for this activation. Of course, in order to achieve a steady state during a continuous prolonged exercise, the rate of ATP synthesis must be equalised with the rate of ATP utilisation. Apart form the first few seconds of exercise, when ATP is supplied mainly from conversion of phosphocreatine (PCr) to creatine (Cr) (reaction catalysed by creatine kinase), and maximal exercise, where anaerobic glycolysis plays a significant role, the main source of ATP in oxidative (type I) skeletal muscle fibres is oxidative phosphorylation. Therefore, the rate of mitochondrial respiration has to be also greatly speeded up in order to meet the increased energy demand during contraction.

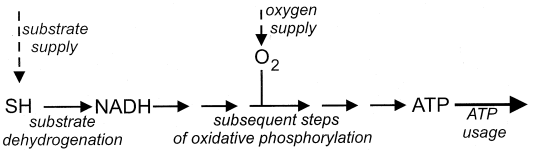

Fig. 1. General scheme of the oxidative phosphorylation system in muscle. The system contains: ATP usage, different steps of oxidative phosphorylation (complex I, complex III, complex IV, ATP synthase, ATP/ADP carrier, phosphate carrier), substrate dehydrogenation, respiratory substrate delivery by blood, oxygen delivery by blood.

Different models of regulatory mechanisms, describing how the oxidative phosphorylation process in mitochondria is 'informed' about the current energy demand, have been proposed. In the present paper, I discuss five of such possible models (three of them have been proposed explicitly in the literature and the remaining two constitute additional theoretical possibilities). I call them: 'output activation', 'input/output activation', 'removal of substrate shortage', 'removal of oxygen shortage' and 'each step activation' (although there are no univocal experimental evidences supporting the removal of substrate shortage and removal of oxygen shortage mechanisms, they are worth discussing as theoretical possibilities). They are presented in Fig. 2 where the same schematic convention as in Fig. 1 is used. It was originally proposed by Chance and Williams [1] that only ATP consumption (output of the system) is activated directly by an external effector, while respiration in mitochondria is activated indirectly, via a decrease in the ATP/ADP ratio (increase in ADP concentration) (output activation). The discovery of the activation in vitro of the irreversible TCA cycle dehydrogenases by calcium ions prompted several authors to postulate that also substrate dehydrogenation (input of the system) is activated directly in parallel with ATP usage (input/output activation) [2,3]. There also exists a theoretical possibility that respiratory substrate concentration in resting state in skeletal muscle, unlike in isolated muscle mitochondria, is very low and to a large extent limiting for the oxygen consumption flux; in such a case, a stimulation of substrate supply by blood, together with the activation of ATP usage, could be sufficient to

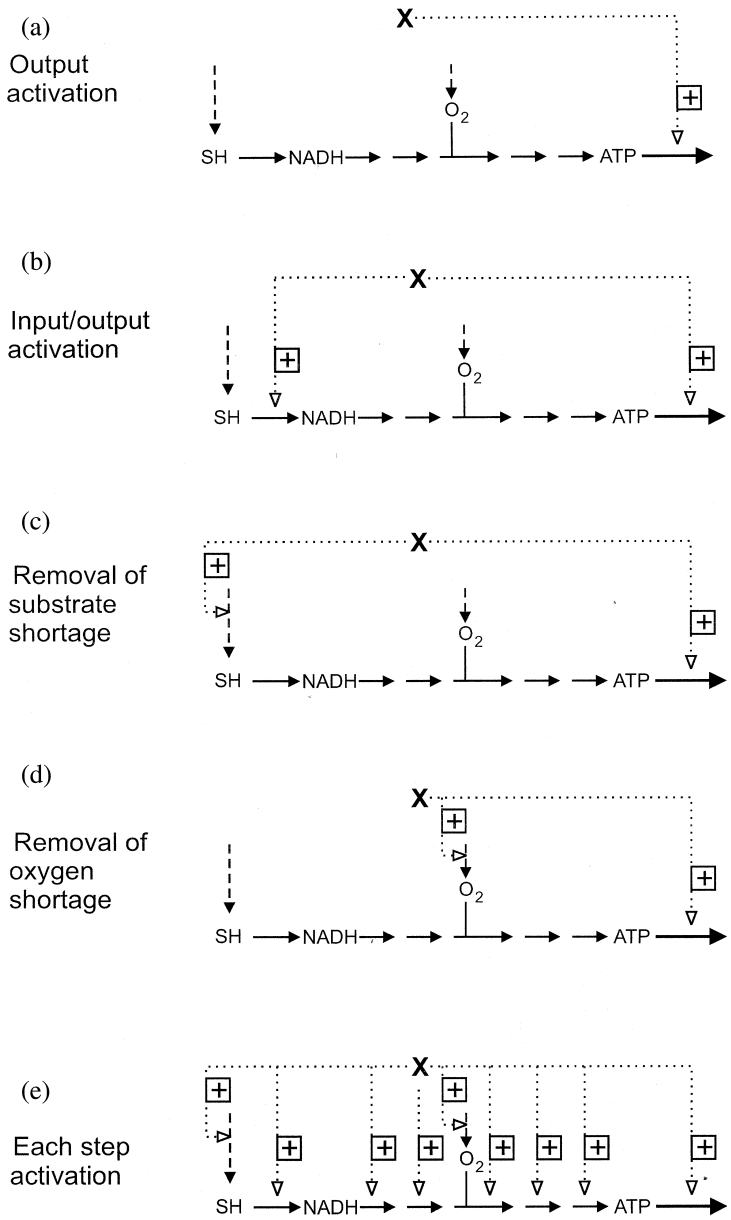

Fig. 2. Five models of the regulation of oxidative phosphorylation. In different models, an external effector *X* activates directly different steps of the system. Details in the text.

cause an appropriate increase in ATP supply (removal of substrate shortage). Similarly, a low oxygen concentration in resting state could be limiting for the flux and an increase in oxygen delivery by blood during work could remove this limitation (removal of oxygen shortage). Finally, the theoretical studies performed with the aid of the dynamic model of oxidative phosphorylation in skeletal muscle mitochondria developed previously [4] lead to the prediction that all the steps of oxidative phosphorylation (enzymes, carriers, blocks of reactions), taken into account explicitly

in this model, should be directly activated to a large extent by an external effector (e.g. calcium ions) (each step activation) [5]. The above models are described in more detail in Section 5.

An analogous problem also exists in other tissues, for example in the case of activation of oxygen consumption and ATP synthesis in heart during an increase of the beating frequency or in liver during activation of oxidative phosphorylation by different hormones, acting via calcium ions (e.g. vasopressin), which speed up the processes of gluconeogenesis and ureagenesis. The accessible experimental data and their theoretical interpretations suggest that also in these cases at least both the ATP-consuming subsystem and ATP-producing subsystem in general (and possibly all particular steps of oxidative phosphorylation in mitochondria) are directly activated by an external effector (e.g. calcium ions) [6–8].

I suggested in the previous article [5] that the parallel activation of each step of oxidative phosphorylation is necessary for an explanation of the changes in the respiration rate and ATP/ADP ratio during transition from resting state to maximal exercise in skeletal muscle. However, it was assumed in that paper that substrate delivery and oxygen delivery by blood were not limiting for the respiration flux and ATP synthesis flux in resting muscle. Therefore, this reasoning was susceptible to the criticism that large relative changes in the respiration rate and relatively low changes in the ATP/ADP ratio in skeletal muscle during resting state → intensive exercise transition were not due to the parallel activation of different steps, but due to a removal of substrate shortage and/or oxygen shortage, simultaneous with the activation of ATP utilisation. In the present debating paper, experimental data which testify against the possibility that a small concentration of respiratory substrate(s) and/or oxygen in resting muscle lowers the respiration rate much below the state 4 respiration level in isolated mitochondria are discussed. Additionally, a much broader range of experimental facts and conceptual models is analysed on a more physiological level. This extended analysis leads to the conclusion that the main mechanism responsible for the adjustment of the rate of ATP synthesis to the rate of ATP consumption in skeletal muscle during intensive exercise is the parallel activation of (almost) all steps of the oxidative phosphorylation system.

2. Selected experimental data

Below, chosen experimental and theoretical results and data, taken from different positions of the literature, are presented. These results are next interpreted and combined in appropriate (i.e. leading to concrete logical conclusions) groups in Section 4 as well as confronted with different models of regulation of oxidative phosphorylation in Section 6. The data concerning the properties of intact skeletal muscle were selected according to three criteria. Firstly, results were taken which concern the transition from resting state to intensive (preferably maximal) exercise in order to involve an as great a range of changes of the respiration rate as possible; as I discussed previously [5], at low work intensities the negative feedback via [ADP] may be the main mechanism of regulation of oxidative phosphorylation. Secondly, a preference was given to the data obtained with neural stimulation of muscle, as more physiological than artificial electrical stimulation (see Section 7). Thirdly, for the same reason, experiments on muscle in vivo rather than on perfused muscle were taken into account. The last choice can be easily justified by the fact that changes in [ADP] during stimulation of heart muscle are much smaller in heart in situ [6] than in perfused heart [9,10].

The selected experimental results are enumerated below:

- Result 1. During transition from resting state to maximal exercise, the respiration rate increases 15–200 times in skeletal muscle of mammals, or even as much as 600 times in flight muscle of insects (see Hochachka [11] for a review). In a very ‘moderate’ case of dog gastrocnemius, taken as the reference point in the previous theoretical studies [5], the respiration rate increases 18 times [12,13].

- Result 2. At the same time, the ATP/ADP ratio decreases two to five times during resting state → maximal exercise transition [11]. In dog gastrocnemius, this ratio decreases approximately three times [12,13].
- Result 3. The respiratory control ratio (RCR, the ratio of the respiration rate in state 3 to the respiration rate in state 4), that is the maximal relative increase in VO_2 caused by changes in the ATP/ADP ratio, is equal up to approximately 10 in isolated skeletal muscle mitochondria [14].
- Result 4. A much greater than 10-fold decrease in the ATP/ADP ratio is needed to cause the increase of the oxygen consumption rate from the state 4 value to the state 3 value [15–18]. A little bit steeper dependence between the respiration and ATP/ADP ratio was reported in Jeneson et al. [19], however, this result was not confirmed by other studies.
- Result 5. The proton leak through the inner mitochondrial membrane accounts for approximately one-half of the respiration rate in resting muscle [20].
- Result 6. During transition form resting state to working state in skeletal muscle, an increase of the $NADH/NAD^+$ ratio is observed in some experiments [21,22], while in other experiments [23–26] this ratio decreases (however, in Godfraind [24] it was reported that the $NADH/NAD^+$ ratio first increases and only then decreases). It is believed that changes in the overall NADH fluorescence correspond at least partially to changes in mitochondrial NADH. Additionally, it seems probable that cytosolic and mitochondrial NAD redox states change in the same direction.
- Result 7. The maximal respiration rate in intact muscle is three to five times greater than the maximal respiration rate in isolated mitochondria (calculated for the same amount of mitochondria) [27,28]. A similar estimation based on data presented in Blei et al. [29] gives an approximate twofold difference in the maximal flux between intact muscle and isolated mitochondria.

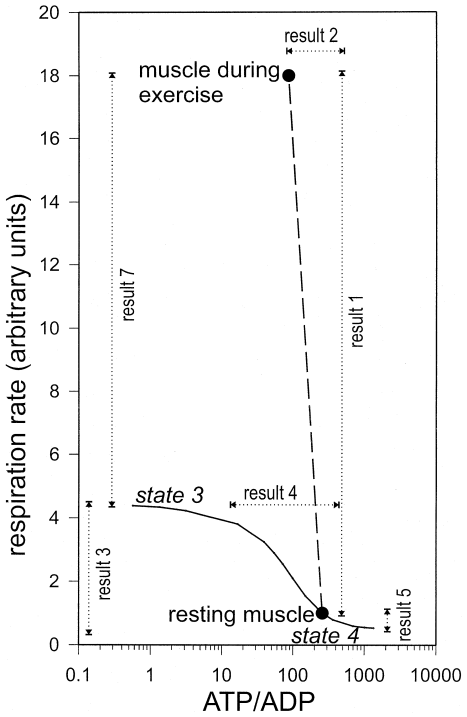

Fig. 3. Graphical presentation of facts 1, 2, 3, 4, 5, 7. The simulated (with an aid of the dynamic computer model of oxidative phosphorylation in muscle mitochondria developed previously) kinetic response of muscle mitochondria to the ATP/ADP ratio (solid line) is compared with the increase in the respiration rate (18-fold) and decrease in the ATP/ADP ratio (threefold) during transition from resting state to intensive exercise in skeletal muscle (dog gastrocnemius in vivo, measured experimentally [9]) (dashed line). Quantitative experimental results 1, 2, 3, 4, 5, 7 described in the text are presented graphically (dotted lines).

Results 1, 2, 3, 4, 5 and 7 are presented graphically in Fig. 3.

3. Previous theoretical results

In the previous paper [5], the kinetic dynamic model of oxidative phosphorylation in muscle mitochondria developed previously [4] was used in theoretical studies on the problem how ATP supply by oxidative phosphorylation in skeletal muscle is regulated during resting state → intensive exercise transition. In this model, each reaction (process) taken into account is described explicitly

Table 1
Kinetic descriptions of the components of oxidative phosphorylation explicitly taken into account in the model

Substrate dehydrogenation:

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

Complex I:

$v_{C1} = k_{C1}\cdot\Delta E_{C1}$

Complex III:

$v_{C3} = k_{C3}\cdot\Delta E_{C3}$

Complex IV:

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

ATP synthase:

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

ATP/ADP carrier:

$$v_{EX} = k_{EX}\cdot\left(\frac{ADP_{fe}}{ADP_{fe}+ATP_{fe}\cdot 10^{-\Psi_e/Z}}-\frac{ADP_{fe}}{ADP_{fi}+ATP_{fi}\cdot 10^{-\Psi_i/Z}}\right)\cdot\left(\frac{1}{1+K_{mADP}/ADP_{fe}}\right)$$

$K_{mADP} = 3.5\ \mu M,$

Phosphate carrier:

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

ATP usage:

$$v_{UT} = k_{UT}\frac{1}{1+\frac{K_{mA}}{ATP_{te}}}$$ $K_{mA} = 15\ \mu M,$

Proton leak:

$v_{LK} = k_{LK1}\cdot(10^{k_{LK2}\cdot\Delta p}-1)$

Adenylate kinase:

$v_{AK} = k_{fAK}\cdot ADP_{fe}\cdot ADP_{me} - k_{bAK}\cdot ATP_{me}\cdot AMP_e$

Direct activation of step A by an external effector: step A is activated n times when its rate constant or maximal velocity is increased n times:

$k_A^1 = k_A^0\cdot n$

or

$V_{Amax}^1 = V_{Amax}^0\cdot n,$

where superscript '1' refers to the state after activation and superscript '0' refers to the state before activation.

by an appropriate kinetic equation, expressing the dependence of the rate of such a reaction on intermediate metabolite concentrations. The kinetic equations appearing in the model are presented in Table 1. Changes in time of concentrations of intermediate metabolites constituting independent variables are described by a set of ordinary differential equations. This set is integrated numerically using a computer. The models have been broadly tested by comparison with experimental data, especially those involving large changes in fluxes and metabolite concentrations [4]. The detailed description of the model is given elsewhere [5].

The theoretical studies performed in Korzeniewski [5] consisted in checking which steps (from among those enumerated in Table 1) should be directly activated by an external effector to cause the changes in the respiration rate and ATP/ADP ratio observed experimentally in skeletal muscle during transition from resting state to maximal work. The rather 'moderate' case of dog gastrocnemius, in which an 18-fold increase in the oxygen consumption as well as a threefold decrease in the ATP/ADP ratio took place, was taken as a reference. In the frame of the model, an activation of a given step by an external effector n times was equivalent to an n-fold increase of the value of the rate constant or maximal velocity of this step (see Table 1). The model is in agreement with experimental results 3 and 4, and the performed simulations take into account experimental results 1, 2, 5 and 7 presented in Fig. 3. The following theoretical results have been obtained in computer simulations:

1. A direct activation of only ATP usage (output activation) cannot account for the changes in V_{O_2} and ATP/ADP observed experimentally in skeletal muscle. The expected (threefold) decrease in the ATP/ADP ratio is obtained when ATP usage is activated 3.3 times, but this causes only a 2.3-fold increase in the respiration rate. On the other hand, when a large (100-fold) activation of ATP usage is imposed, oxygen consumption increases only 4.5 times, while the ATP/ADP ratio drops dramatically more than 1000 times (well below 1) [5].
2. A parallel activation of only ATP usage and substrate dehydrogenation (input/output activation) cannot account for the changes in V_{O_2} and ATP/ADP observed experimentally in skeletal muscle. When ATP usage is activated three times and substrate dehydrogenation is activated 50 times, the expected threefold decrease in the ATP/ADP ratio is observed, but the respiration rate increases only three times. When ATP usage is activated 20 times and substrate dehydrogenation is activated 50 times, the ATP/ADP ratio decreases below 1, while the respiration rate increases only 5.2 times [5].
3. The changes in V_{O_2} and ATP/ADP observed in skeletal muscle during resting state → intensive work transition can be easily explained by a parallel direct activation of ATP usage as well as of all steps of oxidative phosphorylation. The expected increase in the respiration rate (18-fold) and decrease in the ATP/ADP ratio (threefold) are obtained when ATP usage is activated 29 times, while all steps of oxidative phosphorylation but proton leak (that is substrate dehydrogenation, complex I, complex III, complex IV, ATP synthase, ATP/ADP carrier, phosphate carrier) are activated 8.5 times [5].
4. If even one of the above mentioned steps is not directly activated, only approximately a half of the expected increase in the respiration rate takes place, while the ATP/ADP ratio diminishes very significantly below 1 [5].

Therefore, the conclusion was drawn in Korzeniewski [5] that only the each step activation mechanism is able to explain the quoted experimental data. On the other hand, in the cited paper the substrate shortage removal mechanism and oxygen shortage removal mechanism were not analysed. However, I will argue in the present paper that these mechanisms can be excluded on the basis of other experimental data not taken into account in the previous paper (Table 2).

4. Interpretations of results

The above-presented results can be collected in

Table 2
Interpretations and conclusions drown from different combinations of the results discussed in the text

Interpretation of results
Results 1–2 The relative change in V_{O_2} in muscle during transition to maximal exercise is much greater than the relative change in the ATP/ADP ratio, which is in contradiction with a simple Michaelis–Menten kinetics.
Results 1–4 The ratio of the change in V_{O_2} to the change in ATP/ADP is much higher in intact muscle than in isolated mitochondria. This suggests that some mechanisms, which are absent in isolated mitochondria, exist in intact muscle, for example a parallel activation of ATP supply together with ATP usage.
Results 1,3,5 The relative capacity for an increase of oxygen consumption is at least four times greater in intact muscle than in isolated mitochondria.
Result 6 At least in some cases, NADH production is directly activated by an external effector more strongly than NADH consumption.
Result 7 An excellent agreement with the results 1,3,5. This indicates that the much greater relative changes of oxygen consumption in intact muscle than in isolated mitochondria are due to a greater maximal oxygen consumption, and not to a lower minimal oxygen consumption in intact muscle than in isolated mitochondria.

groups and appropriately interpreted. Such groups of results should lead directly to clear, easy to interpret conclusions. Different combinations of results and conclusions drawn on the basis of these results are shown in Table 3. In Section 6, these interpretations are confronted with different models of the regulation of oxidative phosphorylation in response to a changing energy demand.

5. Models

The following, already mentioned, conceptual models of the regulation of oxidative phosphorylation in response to a changing energy demand in skeletal muscle are considered in the present article:

(A) *Output activation*. Within this model, proposed originally by Chance and Williams [1], only

Table 3
Models vs. results: a test of an agreement of the proposed models with the discussed results is presented[a]

Results	Output activation	Input/output activation	Removal of substrate shortage	Removal of oxygen shortage	Each step activation
1–2	−	?	?	?	+
1–4	−	±	±	±	+
1,3,5	−	?	±	±	+
6	−	±	±	−	±
7	−	±	−	−	+
Theoretical results	−	−	n.d.	n.d.	+

[a] +, quantitative agreement; ±, qualitative agreement; ?, lack of contradiction; −, contradiction; n.d., not determined.

ATP utilisation (in the case of skeletal muscle: mainly actinomyosin-ATPase and Ca^{2+}-ATPase) is directly activated by an external effector (in the case of skeletal muscle: calcium ions) (Fig. 2a). The mitochondria as a whole are activated indirectly by an increase in ADP concentration, as well as an increase in P_i concentration and decrease in ATP concentration. Different steps of oxidative phosphorylation within mitochondria are activated via changes in appropriate metabolite concentrations, e.g. particular complexes of the respiratory chain are stimulated by a decrease in the protonomotive force (Δp).

(B) *Input / output activation*. Only two steps of oxidative phosphorylation in mitochondria are directly activated by an external effector (calcium ions), namely ATP usage (output) and some enzymes of substrate dehydrogenation (i.e. irreversible TCA cycle dehydrogenases: pyruvate dehydrogenase, isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase) (input) (Fig. 2b) [2,3]. Other steps are sufficiently active (have a great enough capacity) and sensitive to intermediate metabolite concentrations to adjust passively their rates to changes in the overall flux.

(C) *Removal of substrate shortage*. In resting muscle, substrate concentration is much below the saturating level and rate-limiting. Therefore, resting respiration in muscle is much below state 4 respiration in isolated mitochondria. Only ATP usage and substrate delivery by blood are directly activated during an onset of muscle exercise, while other steps are activated indirectly, via changes in intermediate metabolite concentrations (Fig. 2c).

(D) *Removal of oxygen shortage*. In resting muscle, oxygen concentration is much below the saturating level and rate-limiting. Therefore, resting respiration in muscle is much below state 4 respiration in isolated mitochondria. Only ATP usage and oxygen delivery by blood are directly activated during an onset of muscle exercise, while other steps are activated indirectly, via changes in intermediate metabolite concentrations (Fig. 2d).

(E) *Each step activation*. Each step of the oxidative phosphorylation system taken into account explicitly in the dynamic computer model of oxidative phosphorylation developed previously [4,5] is directly activated to a large extent (of the order of 10 times or even more) by an external effector (Fig. 2e). The relevant steps are: ATP usage, substrate dehydrogenation (e.g. tricarboxylate acid cycle dehydrogenases), particular enzymes of oxidative phosphorylation (complex I, complex III, complex IV, ATP synthase, ATP/ADP carrier, phosphate carrier), substrate delivery by blood, oxygen delivery by blood.

6. Models vs. results

In Table 3, the presented conceptual models are confronted with the chosen (combinations of) results taken from the literature. It can be seen that output activation model is in contradiction with all the experimental results discussed in the present paper. Generally, it cannot explain large changes in the respiration flux accompanied by very moderate changes in the ATP/ADP ratio, a greater capacity for the oxygen consumption flux of mitochondria in vivo than in vitro, an increase in the $NADH/NAD^+$ ratio during exercise observed at least in some experiments as well as it is not consistent quantitatively with simulations made using the dynamic computer model of oxidative phosphorylation. The input/output activation model is apparently not contradictory (at least qualitatively) with the existing experimental data. However, a more detailed quantitative analysis with an aid of the dynamic computer model of oxidative phosphorylation shows that this mechanism is not able to explain the changes in the respiration rate and the ATP/ADP ratio taking place during transition from resting state to maximal exercise [5]. Namely, the input/output activation model does not take into account the fact that the capacities and sensitivities to intermediate metabolite concentrations of different mitochondrial enzymes are too small to adjust passively the rates of the reactions catalysed by these enzymes to a great increase in the overall flux. Therefore, the quantitative theoretical treatment proves in this case that a qualitative explanation, which is acceptable intuitively does not work when quantitative properties of the considered system are taken into account. The removal of the substrate shortage model evidently

cannot be reconciled with the fact that the maximal capacity of oxidative phosphorylation in muscle is much greater than in isolated mitochondria (result 7). The removal of oxygen shortage model is inconsistent not only with result 7, but also with the increase in the $NADH/NAD^+$ ratio during muscle contraction observed at least in some experiments (result 6). Only the each step activation model exhibits a very good quantitative agreement with all the experimental results and theoretical studies discussed in the present paper. This suggests that the direct activation by an external effector of each step of oxidative phosphorylation (taken into account explicitly in the discussed dynamic computer model of this process) is the main mechanism responsible for the adjustment of the rate of ATP synthesis to the current energy demand in skeletal muscle during transition from resting state to intensive exercise.

7. Discussion

The present paper tries to resolve the problem as to how ATP production by oxidative phosphorylation meets ATP demand at an onset of an intensive exercise in skeletal muscle. More precisely, it deals with the sub-problem which steps of the bioenergetic system of a muscle cell, especially of oxidative phosphorylation in mitochondria, must be directly activated by an external effector to cause the changes in the respiration rate and ATP/ADP ratio (and also in other metabolite concentrations) observed experimentally during resting state → maximal work transition in skeletal muscle.

Several possible models are analysed by their comparison with selected experimental data. Only the each step activation mechanism is able to explain in a quantitative way the changes in fluxes and metabolite concentrations taking place in intact muscle, especially when they are referred to the changes occurring in isolated mitochondria during state 4 → state 3 transition.

The fact that the output activation mechanism fails completely as the main explanation of the accessible experimental data becomes intuitively obvious when we consider the hyperbolic kinetics of mitochondria with the apparent Michaelis–Menten constant for [ADP] equal to approximately 25 μM, on one hand, and the changes in the respiration rate and [ADP] (ATP/ADP ratio) which take place in intact muscle, on the other hand. One could argue that not only ADP concentration, but also P_i concentration changes significantly in intact muscle during resting state → active state transition. On the other hand, $[P_i]$ is essentially constant in suspension of isolated mitochondria during state 4 → state 3 transition. Therefore, the maximal 10-fold increase in the respiration rate in response to changes in the ATP/ADP ratio (the RCR ratio fixing the capacity of mitochondria for an increase of the oxygen consumption flux) observed in isolated mitochondria cannot be extrapolated to intact muscle conditions. For example, because of the presence in intact muscle of creatine kinase and the PCr/Cr pair which buffers the ATP/ADP ratio, the concentration of inorganic phosphate can increase several times during transition to intensive exercise [29]. Therefore, the computer dynamic model based on experiments performed on isolated mitochondria cannot reflect properly the bioenergetic properties of the intact muscle cell. However, when a saturating concentration of external inorganic phosphate is introduced in state 3 within the computer model of oxidative phosphorylation, the maximal capacity of oxidative phosphorylation for a relative increase in the respiration flux increased from approximately 10 times to only approximately 12–13 times. This suggests that changes in $[P_i]$ (accompanying changes in [ADP]) cannot stimulate oxidative phosphorylation much stronger than the changes in [ADP] alone do ([ATP] remains essentially constant even in intact muscle). This is because the kinetic effects of ADP and P_i do not add linearly, which can be easily understood in terms of the two-substrate enzyme kinetics (mitochondria are more than half-saturated with $[P_i]$ even in resting state). Therefore, an involvement of the changes in inorganic phosphate which occur in intact muscle cannot increase signifi-

cantly the capacity of mitochondria for an increase in the respiration (and ATP synthesis) rate in response to an elevated energy demand.

Some studies [30–32] showed a near-hyperbolic or near-linear relationship between ADP concentration and the ATP synthesis rate or respiration rate during muscle exercise. However, these studies were performed for relatively very low work intensities, covering up to 10% of the maximal capacity for ATP turnover [11]. Additionally, the electrical stimulation of muscle used in these studies (contrary to the physiological neural stimulation) can cause some non-physiological effects, for example non-specific stimulation of glycolysis (e.g. by a recruitment of glycolytic fibres), or even a damage of fibres (especially at higher stimulation frequencies). This can in turn cause a non-physiological decrease in the ATP/ADP ratio as well as in the maximal capacity of oxidative phosphorylation for an increase in the oxygen consumption flux. For the purpose of studies on the regulation of oxidative phosphorylation, it seems better to measure the oxygen consumption flux instead of the ATP synthesis flux (elimination of glycolytic ATP supply) and to use a neural stimulation instead of electrical stimulation (avoiding a non-physiological increase in ADP). Nevertheless, the mentioned experiments can testify to this that the negative feedback via [ADP] is actually the main mechanism which stimulates ATP production by mitochondria at low work intensities, while at medium and high work intensities the parallel direct activation of different steps of oxidative phosphorylation by an external effector is recruited and starts to predominate [5] (the negative feedback via [ADP] remains a minor, fine-tuning mechanism).

There have also been proposed some variants of the output activation model, where a negative feedback via creatine concentration, rather than via ADP concentration, is involved. Mahler [33] drew attention to the fact that there exists a linear relationship between the respiration rate and creatine concentration during muscle contraction. On the basis of this observation, the author concluded that oxidative phosphorylation is directly activated by [Cr] and that this process can be described by a simple first-order kinetics. Leaving aside the fact that a linear correlation does not imply a direct causal relationship, only absolute changes in the respiration rate and creatine concentration were considered in the mentioned paper. When relative changes in these parameters are taken into account, it can be easily calculated (see Fig. 6 and resting [Cr] quoted in Mahler [33]) that a 30-fold increase in the respiration rate is accompanied by an only threefold increase in creatine concentration. It remains a puzzle, how this fact can be reconciled with the first-order kinetics postulated by the author. On the other hand, the presented data can be easily explained within the paradigm of a parallel direct stimulation of different steps of oxidative phosphorylation by an external effector.

The observation that the affinity of mitochondria to ADP in skinned fibres of cardiac muscle and slow-twitch skeletal muscle increases dramatically in the presence of creatine, to the value observed in isolated mitochondria [34], was interpreted by the authors as evidence for an existence of an ADP diffusion limitation and/or displacement from equilibrium of creatine kinase. However, these experiments only show that some structural limitation for ADP diffusion (e.g. micro-organisation or channelling) is present in skinned fibres, and that this limitation is removed after an addition of creatine, and therefore after a restoration of more physiological conditions. In this aspect, isolated mitochondria (no diffusion limitation because of a destruction of the postulated structural micro-compartmentation between mitochondrial creatine kinase (mi-CK) and ATP/ADP carrier) are a better model for studies of the kinetic properties of oxidative phosphorylation in intact muscle (no diffusion limitation because of the presence of creatine, which overcomes ADP diffusion limitations caused by micro-compartmentation) than skinned fibres without creatine (diffusion limitations present, because the micro-organisation is preserved, while creatine is lacking). (Of course, the system: skinned fibres + creatine is an equally good, if not better, model of oxidative phosphorylation in intact muscle as isolated mitochondria.) This conclusion is strongly supported by the fact that many experiments performed on intact muscle

testify against any diffusion limitations for ADP [35,36]. Moreover, Aliev and Saks [37] developed a mathematical model of oxidative phosphorylation in cardiac muscle which involves the postulated diffusion limitations as well as a displacement from equilibrium of creatine kinase, in order to explain the very small changes in ADP concentration observed experimentally during a several-fold increase in oxygen consumption in the heart [6]. They argued that just the postulated compartmentalised energy transfer in cardiomyocytes is responsible for small changes in bulk-phase [ADP]. However, paradoxically, just their own model shows excellently the need for a parallel activation of ATP supply together with ATP usage. Because, the simulations performed by the authors gave a twofold decrease in the PCr/ATP ratio during an approximate fivefold increase of the phosphorylation flux (Figure 14B in Aliev and Saks [37]), while essentially no changes in this ratio were observed experimentally in heart at a comparable change in the flux [6]. Again, this discrepancy can be easily explained on the grounds of the concept of parallel activation of ATP consumption and ATP production. Additionally, Kushmerick [38], using experimentally-measured kinetic properties of creatine kinase, showed that this enzyme is near equilibrium in muscle even at high flux intensities.

One could also argue that the process of oxidative phosphorylation in intact muscle, unlike in isolated mitochondria, exhibits a property of ultrasensitivity of the flux of respiration (and ATP synthesis) to changes in the ATP/ADP ratio (and also is characterised by a greater capacity for an increase in these fluxes). For this reason, the output activation mechanism + negative feedback via ADP concentration still remains a valid explanation of the existing experimental data. However, this supposition would imply that the properties of isolated mitochondria are completely different than the properties of mitochondria in situ. There are no reasons to accept such an assumption. Moreover, Brand, Brown and colleagues clearly demonstrated, using the 'top-down' approach to Metabolic Control Analysis, that the kinetic properties of oxidative phosphorylation in isolated liver mitochondria [39] are very similar to the properties of oxidative phosphorylation in intact hepatocytes [40]. In both cases, a comparable contribution of the proton leak to the overall oxygen consumption flux as well as a similar distribution of metabolic control among the oxidation system, phosphorylation system and proton leak system were stated, which testifies that mitochondria were not damaged during isolation and their properties were not changed. This conclusion can be reasonably extrapolated to oxidative phosphorylation in skeletal muscle, especially that kinetic properties of muscle mitochondria do not differ by anything significant from the kinetic properties of liver mitochondria [14,41]. Similar kinetic properties of oxidative phosphorylation were also observed in skinned fibres when ATP consumption (actinomyosin ATPase) was stimulated by calcium ions [42]. These findings greatly validate the theoretical result that the discussed dynamic computer model of oxidative phosphorylation, successfully tested for virtually all known kinetic properties of oxidative phosphorylation in isolated mitochondria, decidedly excludes the possibility that the negative feedback via [ADP] is the main mechanism responsible for an adjustment of ATP production to current energy demand at high exercise intensities [5].

Additionally, the output activation mechanism (also in the 'ultrasensitive' version) is not able to explain the increase in the $NADH/NAD^+$ ratio after an onset of muscle contraction observed in some experiments. The fact that the $NADH/NAD^+$ ratio increases during muscle work in same cases [21,22], while it decreases in other cases [23–26] testifies to this that there exists a very subtle balance of activation on the NADH-production side and on the NADH-consumption side. Indeed, relatively small changes in the degree of activation by an external effector of the NADH-producing block (substrate dehydrogenation) and the NADH-consuming block (respiratory chain) within the dynamic computer model of oxidative phosphorylation gave either a quite high increase in the $NADH/NAD^+$ ratio after transition to active state, or a decrease in this ratio [data not shown]. Therefore, the fact that the reduction level of NAD decreases in some cases after an onset of muscle contraction by no means

testifies univocally to the validity of the output activation mechanism.

Finally, a very recent work by Rolfe et al. [43] shows that the protonmotive force does not change or even slightly increases during stimulation of muscle. Again, this result can be explained only in the frame of the paradigm of parallel direct activation of the Δp-producing block and Δp-consuming block. When the proportional activation approach [8] is used to elaborate quantitatively these data, one can easily calculate for this case the value of the proportional activation coefficient (defined as the ratio of the relative stimulation of the Δp-consuming block (B) to the relative stimulation of the Δp-producing block (A) by an external effector X: $P_X^{AB} = \partial B/B/\partial A/A$, where A and B stand for the activities of the two blocks). This value equals roughly 0.75, which means that Δp consumption was activated by only 25% less than Δp production.

At the first look, the input/output activation mechanism seems to be intuitively logical and to offer an attractive qualitative explanation of the experimental results discussed in this paper. Nevertheless, this mechanism does not work when more quantitative analysis of the system is performed. This analysis involves maximal capacities of particular steps for an increase in the flux as well as sensitivities of these steps to intermediate metabolite concentrations. It also takes into account the fact that flux control coefficients of substrate dehydrogenation and particular membrane complexes of oxidative phosphorylation are of the same order of magnitude [14] and therefore all these steps become saturated more or less simultaneously when the flux increases. In order to draw final conclusions, all these facts should be analysed in a quantitative way. Because the human brain cannot cope with such an analysis, a quantitative treatment with the aid of well-tested computer models plays a crucial role in these kinds of studies. Such a treatment performed in the previous paper [5] suggests that the input/output activation mechanism cannot account for the experimentally observable behaviour of the investigated system.

The substrate shortage removal mechanism is contradictory not only with the fact that the maximal respiration in intact muscle is two to five times greater than in isolated mitochondria (result 7). Because, a significant substrate shortage in resting state, and therefore a considerable decrease in the ATP supply rate as well as in the phosphorylation potential, would hinder many reactions driven by ATP hydrolysis, important for keeping a cell alive, e.g. protein synthesis, DNA and RNA synthesis, ion (Na^+, K^+) circulation and so on [44]. Finally, the phosphorylation potential measured in resting muscle [35,45] is at least as high (if not higher) as the phosphorylation potential in isolated mitochondria in the 'physiological' state 3.5 (a state intermediate between state 4 and state 3) [15–18]. For all these reasons, the removal of substrate shortage mechanism does not seem to reflect correctly the situation (regulatory mechanisms) which actually takes place in intact muscle.

Some support for the oxygen shortage removal mechanism can be given by the fact that Gneiger et al. [46] found that the oxygen concentration in heart muscle reported in some publications is greater by 'only' approximately one order of magnitude than the K_m constant of oxidative phosphorylation for oxygen concentration measured by them. The authors concluded that this oxygen concentration is located in the 'control range' for oxidative phosphorylation, since it lowers the respiration rate to approximately 90% of the value expected at saturating oxygen concentration. However, this means nothing more than that an increase of oxygen delivery by blood can, in itself, increase the respiration rate by only up to 10%. On the other hand, an approximate 10-fold (by 900%) activation of oxidative phosphorylation is necessary to explain the changes in fluxes and metabolite concentrations observed experimentally during muscle contraction [5]. Therefore, an activation of oxygen delivery by blood does not seem to be a significant mechanism responsible for the stimulation of respiration rate and ATP synthesis during transition from the resting state to intensive exercise. Of course, the supply of substrate and oxygen by blood must be increased during muscle contraction, however, not in order to activate the oxidative phosphorylation system

in resting state, but to avoid a decrease in oxygen (and substrate) concentration and therefore an inhibition of this system in active state. (This does not have to lead to an increase in oxygen concentration if oxygen supply and oxygen consumption are activated to the same extent.) In other words, the oxygen concentration does not control significantly the respiration flux; inversely, just this concentration is controlled by the balance between the rate of oxygen consumption and the rate of oxygen delivery by blood. Additionally, the oxygen shortage removal mechanism cannot be reconciled with result 6 and the whole criticism concerning the removal of substrate shortage mechanism applies equally well to the removal of the oxygen shortage mechanism.

Taking into account the above discussion, a conclusion can be drawn that only the 'each step' activation mechanism agrees in a satisfactory, quantitative way with the known facts and computer simulations based on the model of oxidative phosphorylation in muscle mitochondria developed previously. This suggests that (a) universal intracellular activator(s)/regulatory mechanism(s) of different steps must exist. However, the main known candidate, namely calcium ions, is not fully satisfactory because their action in vitro is too small and/or doubtful [47,48]. The computer model predicts that all steps of oxidative phosphorylation should be activated to a large extent (10 times or so). On the other hand, it still remains a matter of debate if calcium activates some complexes in mitochondria in vivo. Even in these few cases where it is proposed that calcium activates some enzymes, the extent of stimulation is decidedly too small to account quantitatively for the phenomena taking place in skeletal muscle (for example a less than twofold up-regulation of ATP synthase in heart [49]). Therefore, one can expect that our present knowledge lacks something very significant, an important element necessary for explanation of how ATP supply meets ATP demand. This would mean that a general effector/regulatory mechanism is still waiting for experimental identification. In this sense, the simulations performed previously [5] with an aid of the computer dynamic model of oxidative phosphorylation and discussed in this article may inspire and direct further experimental studies. At the present stage, one can speculate on the physical nature of the theoretically predicted universal effector/regulatory mechanism.

Considering the activation by calcium ions of TCA cycle dehydrogenases in vitro and some effect of calcium on some complexes of oxidative phosphorylation [2,3,47,48], it seems probable that this universal effector has something to do with calcium ions. However, for the reasons mentioned before, a high $[Ca^{2+}]$ alone does not seem to be the obvious factor. I proposed in the previous article [5] that calcium ions may act via some protein, analogous to calmodulin, which is lost or inactivated during isolation of mitochondria and especially during isolation of particular enzymes. This possibility can be supported by the fact that, e.g. 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 [50]. This suggests that something mediates in 'presenting' calcium ions to the ATP/ADP carrier. Such a protein could be responsible for the integration over time of the signal coming from the frequency of Ca^{2+} oscillations (see below).

Another (alternative or supplementary) possibility comes from calcium spiking discovered in hepatocytes. It was demonstrated [51] that hormones coupled with a release of calcium ions to cytoplasm (e.g. vasopressin, phenylephrine) which activate respiration and ATP synthesis, generate periodic $[Ca^{2+}]$ spikes in the cytoplasm. It was subsequently shown [52] that cytosolic calcium spikes are reflected in oscillations in mitochondrial calcium ion concentrations occurring in the same phase. The frequency of spiking was integrated over time, which manifested itself in a persistently elevated reduction level of NAD and protonmotive force [52]. This suggests that calcium spiking causes a frequency-dependent activation of at least some mitochondrial enzymes. On the other hand, paradoxically, a high constant cytosolic concentration of Ca^{2+} (present at high doses of hormones) caused only a transient increase in mitochondrial calcium concentration and, as a result of this, as a transient activation of

oxidative phosphorylation. This could well explain why isolated mitochondria are activated relatively weakly, or, not at all, by high constant external concentrations of calcium ions [48]. The frequency-dependent stimulation of oxidative phosphorylation in mitochondria by calcium oscillations seems to be an attractive candidate for the postulated universal mechanism which activates in parallel all the complexes of oxidative phosphorylation in skeletal muscle in order to adjust the rate of ATP synthesis to current energy demand.

The postulated each step activation mechanism is, in an important sense, a logical derivative of a theoretical and experimental development of the Metabolic Control Analysis [53,54]. Because, this method demonstrated that the metabolic control is distributed among many (virtually all) steps of different metabolic pathways, especially of oxidative phosphorylation [14,41]. Additionally, in a typical case, if one step is activated by an external effector, the flux control coefficients of the remaining steps increase. Therefore, all steps should be directly activated to cause a large increase of the metabolite flux through a considered biochemical pathway. On the other hand, if the parallel activation of different steps actually takes place, the values of flux control coefficients of particular enzymes loose to a large extent their importance, because in the case of an ideally equal activation of each step by n times, the overall flux will also increase n times regardless of the values of flux control coefficients of particular enzymes. In the case of a non-ideally equal activation, which occurs in the bioenergetic system of skeletal muscle (ATP usage is activated more than ATP supply), the values of flux control coefficient of particular steps are relevant for the 'fine tuning' mechanism of equilibration of different reaction rates via moderate changes in intermediate metabolite concentrations.

The each step activation mechanism, if correct, completely changes the traditional paradigm of the regulation of the bioenergetic metabolism, based on the negative feedback loop involving changes in [ADP] and other metabolite concentrations. Two possible evolutionary profits of the parallel activation of different steps can be considered. Firstly, it allows to maintain a relatively constant concentration of different intermediate metabolites, such as ATP, NADH, Δp and acetyl-CoA. These metabolites are substrates for many anabolic and transport processes important for the function and survival of a cell. A significant decrease in the concentrations of these metabolites could stop the mentioned processes and lead to damage or even death of a cell. Secondly, the each step activation mechanism would enable to keep the concentrations of the relevant enzymes low and therefore to diminish energy expanses for protein synthesis. Because, in the absence of a direct activation, the concentration of a given enzyme would have to be significantly greater in order to achieve the large desired maximal capacity for an increase of a flux.

Summing up, the each step activation proposal discussed in the present article seems to be the only quantitatively-working interpretation of the accessible experimental data as well as a logical consequence of the homeostasis of the cell conditioned by the purposefulness of biological evolution. A similar idea, named 'multi-site modulation', was developed in a more abstract and general way by Fell and Thomas [55] in relation to other pathways, especially glycolysis.

Acknowledgements

This work was supported by the KBN grant. I am grateful to Jerzy Zoladz for a stimulating discussion.

References

[1] B. Chance, G.R. Williams, J. Biol. Chem. 217 (1955) 383–393.
[2] R.M. Denton, J.G. McCormack, FEBS Lett. 119 (1980) 1–8.
[3] R.M. Denton, J.G. McCormack, Biochim. Biophys. Acta 1018 (1990) 278–291.
[4] B. Korzeniewski, J.-P. Mazat, Biochem. J. 319 (1996) 143–148.
[5] B. Korzeniewski, Biochem. J. 330 (1998) 1189–1195.
[6] R.S. Balaban, H.L. Kantor, L.A. Katz, R.W. Briggs, Science 232 (1986) 1121–1123.
[7] B. Korzeniewski, W. Froncisz, Biochim. Biophys. Acta 1102 (1992) 67–75.

[8] B. Korzeniewski, M.-E. Harper, M.D. Brand, Biochim. Biophys. Acta 1229 (1995) 315–322.
[9] F.M.H. Jeffrey, C.R. Malloy, Biochem. J. 287 (1992) 117–123.
[10] A.H.L. From, S.D. Zimmer, S.P. Michurski et al., Biochemistry 29 (1990) 3731–3743.
[11] P.W. Hochachka, Muscles as Molecular and Metabolic Machines, CRC Press, Boca Raton, 1994.
[12] M.C. Hogan, P.G. Arthur, D.E. Bebout, P.W. Hochachka, P.D. Wagner, J. App. Physiol. 73 (1992) 728–736.
[13] P.G. Arthur, M.C. Hogan, P.D. Wagner, P.W. Hochachka, J. Appl. Physiol. 73 (1992) 737–743.
[14] T. Lettelier, M. Malgat, J.-P. Mazat, Biochim. Biophys. Acta 1141 (1993) 58–64.
[15] E.A. Wanders, H.V. Westerhoff, Biochemistry 27 (1988) 7832–7840.
[16] J.M. Tager, R.J.A. Wanders, A.K. Groen et al., FEBS Lett. 151 (1983) 1–9.
[17] A. Holian, C.S. Owen, D.F. Wilson, Arch. Biochem. Biophys. 181 (1977) 161–171.
[18] R. Bohnensack, F.N. Gellerich, L. Schild, W. Kunz, Biochim. Biophys. Acta 1018 (1990) 182–184.
[19] J.A.L. Jeneson, R.W. Wiseman, H.V. Westerhoff, M.J. Kushmerick, J. Biol. Chem. 271 (1996) 27995–27998.
[20] D.F.S. Rolfe, M.D. Brand, Am. J. Physiol. 271 (1996) C1380–C1389.
[21] D. Duboc, M. Muffat-Joly, G. Renault, M. Degeorges, M. Toussaint, J.-J. Pocidalo, J. App. Physiol. 64 (1988) 2692–2695.
[22] K. Sahlin, Pflug. Arch. 403 (1985) 193–196.
[23] F.F. Jöbsis, W.N. Stainsby, Respir. Physiol. 4 (1968) 292–300.
[24] A. Godfraind, J. Physiol. 223 (1972) 719–734.
[25] J.B. Chapman, J. Gen. Physiol. 59 (1972) 135–154.
[26] I.R. Wendt, J.B. Chapman, Am. J. Physiol. 230 (1976) 1644–1649.
[27] M. Tonkonogi, K. Sahlin, Acta Physiol. Scand. 161 (1997) 345–353.
[28] P. Andersen, B. Saltin, J. Physiol. 366 (1985) 233–249.
[29] M.L. Blei, K.E. Conley, M.J. Kushmerick, J. Physiol. 465 (1993) 203–222.
[30] M.J. Kushmerick, R.A. Meyer, T.R. Brown, Am. J. Physiol. 263 (1992) C598–C606.
[31] R.A. Meyer, J.M. Foley, Med. Sci. Sports Exerc. 26 (1994) 52–57.
[32] K.M. Brindle, M.J. Blackledge, R.A.J. Calliss, G.K. Radda, Biochemistry 28 (1989) 4887–4893.
[33] M. Mahler, J. Gen. Physiol. 86 (1985) 135–165.
[34] L. Kay, Z. Li, M. Mericskay et al., Biochim. Biophys. Acta 1322 (1997) 41–59.
[35] E.W. McFarland, M.J. Kushmerick, T.S. Moerland, Biophys. J. 67 (1994) 1912–1924.
[36] R.W. Wiseman, M.J. Kushmerick, J. Biol. Chem. 270 (1995) 12428–12438.
[37] M.K. Aliev, V.A. Saks, Biophys. J. 73 (1997) 428–445.
[38] M.J. Kushmerick, Comp. Biochem. Physiol. 120 (1998) 109–123.
[39] R.P. Hafner, G.C. Brown, M.D. Brand, Eur. J. Biochem. 188 (1990) 313–319.
[40] G.C. Brown, P.L. Lakin-Thomas, M.D. Brand, Eur. J. Biochem. 192 (1990) 355–362.
[41] A.K. Groen, R.J.A. Wanders, H.V. Westerhoff, R. van der Meer, J.M. Tager, J. Biol. Chem. 257 (1982) 2754–2757.
[42] E. Wisniewski, F.N. Gellerich, W.S. Kunz, Eur. J. Biochem. 230 (1995) 549–554.
[43] D.F. Rolfe, J.M.B. Newman, J.A. Buckingham, M.G. Clark, M.D. Brand, Am. J. Physiol. 276 (1999) C692–C699.
[44] G.C. Brown, Biochem. J. 284 (1992) 1–13.
[45] T. Walliman, M. Wyss, D. Brdiczka, K. Nicolay, H.M. Eppenberger, Biochem. J. 281 (1992) 21–40.
[46] E. Gneiger, R. Steinlechner-Maran, G. Méndez, T. Eberl, R. Margreiter, Bioenerg. Biomembr. 27 (1995) 583–596.
[47] R. Moreno-Sanchez, B.A. Hogue, R.G. Hansford, Biochem. J. 268 (1990) 421–428.
[48] V. Mildaziene, R. Baniene, Z. Nauciene et al., Biochem. J. 320 (1996) 329–334.
[49] D.A. Harris, A.M. Das, Biochem. J. 280 (1991) 561–573.
[50] R. Krämer, U. Mayr, C. Heberger, S. Tsompanidou, Biochim. Biophys. Acta 855 (1996) 201–210.
[51] N.N. Woods, K.S. Cuthberston, P.H. Cobbold, Nature 319 (1986) 600–602.
[52] L.D. Robb-Gaspers, G.A. Rutter, P. Burnett, G. Hajnóczky, R.M. Denton, A.P. Thomas, Biochim. Biophys. Acta 1366 (1998) 17–32.
[53] H. Kacser, J.A. Burns, Symp. Soc. Exp. Biol. 32 (1973) 61–104.
[54] R. Heinrich, T.A. Rapoport, Eur. J. Biochem. 42 (1994) 89–95.
[55] D.A. Fell, S. Thomas, Biochem. J. 311 (1995) 35–39.