Training-induced adaptation of oxidative phosphorylation in skeletal muscles

Bernard KORZENIEWSKI*¹ and Jerzy A. ZOLADZ†

*Institute of Molecular Biology and Biotechnology, Jagiellonian University, ul. Gronostajowa 7, 30-387 Kraków, Poland, and †Department of Muscle Physiology, AWF-Kraków, al. Jana-Pawła II 78, 31-571 Kraków, Poland

Muscle training/conditioning improves the adaptation of oxidative phosphorylation in skeletal muscles to physical exercise. However, the mechanisms underlying this adaptation are still not understood fully. By quantitative analysis of the existing experimental results, we show that training-induced acceleration of oxygen-uptake kinetics at the onset of exercise and improvement of ATP/ADP stability due to physical training are mainly caused by an increase in the amount of mitochondrial proteins and by

an intensification of the parallel activation of ATP usage and ATP supply (increase in direct stimulation of oxidative phosphorylation complexes accompanying stimulation of ATP consumption) during exercise.

Key words: ATP/ADP homoeostasis, computer model, oxygen-uptake kinetics, parallel activation, regulation of metabolism.

INTRODUCTION

The efficiency of regulation of the ATP production system in skeletal muscles may be characterized quantitatively by the duration of transition of metabolic fluxes and metabolite concentrations between rest and work and by the stability of intermediate metabolite concentrations, especially of the ATP/ADP ratio.

During the transition from rest to work, the rate of ATP supply must be rapidly increased to meet the high rate of ATP consumption set by the intensity of muscle contraction. At low and moderate exercise intensity, the conversion of phosphocreatine (PCr) into creatine, catalysed by creatine kinase, is the main process supplying ATP during the first tens of seconds of exercise; later, ATP production is gradually taken over by oxidative phosphorylation, which is expressed as an intensification of oxygen consumption. At the onset of exercise, oxygen uptake/min, $\dot{V}O_2$, increases from rest to work in a near-exponential way. This transition is characterized quantitatively by the transition time $t_{1/2}$ or τ .

The stability of intermediate metabolite concentrations, such as ATP/ADP, proton-motive force Δp , NADH/NAD⁺ or acetyl-CoA, is critical for cell homoeostasis, because large changes in these concentrations could disturb several reactions/processes, the rates of which depend on the above-mentioned metabolite concentrations. Therefore any mechanism to improve the stability of metabolite concentrations is profitable for muscle functioning.

It has been demonstrated that training/conditioning of muscles not only shortens the transition time of $\dot{V}O_2$ kinetics significantly [1–5] but also decreases the relative changes in [ADP] (ATP/ADP ratio) between rest and work for a given increase in oxygen consumption (work intensity) [6–8]. Several studies have shown a training-induced increase in mitochondria/mitochondrial proteins [9–13]. A recent study demonstrated that training may decrease the K_m value of mitochondria for ADP [14]. Both effects have been suggested to increase the sensitivity of oxidative phosphorylation to ADP [7,8,14,16] and thus improve the stability of ADP during muscle contraction. Also, these effects may be potentially responsible for a training-induced decrease in $t_{1/2}$ values of $\dot{V}O_2$ kinetics.

Intensification of the parallel activation of ATP supply and ATP demand (direct activation of different oxidative phosphorylation

complexes in parallel with the activation of ATP usage) during muscle contraction [15] can be another possible explanation for training-induced acceleration of $\dot{V}O_2$ kinetics and for the improvement of [ADP] stability. It has been demonstrated, with the aid of the computer model of oxidative phosphorylation in muscle mitochondria [17], that only the parallel-activation mechanism is able to explain the minute relative changes in [ADP] accompanying large relative changes in $\dot{V}O_2$ during the transition from rest to work [15].

Using a previously developed computer model of oxidative phosphorylation in intact skeletal muscles [18] and simple quantitative analysis not requiring the model, the present study aims to resolve which of the following mechanisms is responsible for training-induced shortening of $t_{1/2}$ of $\dot{V}O_2$ kinetics and for improvement of the ATP/ADP homoeostasis: increase in mitochondria/mitochondrial proteins, decrease in the K_m value of oxidative phosphorylation for ADP and/or intensification of parallel activation.

THEORETICAL PROCEDURES

A previously developed computer model of oxidative phosphorylation in intact skeletal muscles [18] was used in the present theoretical study. The following enzymes, processes and metabolic blocks are taken into account explicitly within the model: substrate dehydrogenation (hydrogen supply to the respiratory chain including tricarboxylic acid cycle, glycolysis, glycogenolysis, glucose transport, fatty acid β -oxidation, fatty acid transport and so on), complexes I, III and IV (cytochrome *c* oxidase), proton leak, ATP synthase, ATP/ADP carrier, phosphate carrier, adenylate kinase, creatine kinase and ATP usage. Time variations of the metabolite concentrations that constitute independent variables (NADH, ubiquinol, reduced form of cytochrome *c*, O_2 , internal protons, internal ATP, internal P_i , external ATP, external ADP, external P_i , external protons and PCr) are expressed in the form of a set of ordinary differential equations. The other (dependent) variables (other metabolite concentrations, thermodynamic forces, etc.) are calculated from the independent

Abbreviation used: PCr, phosphocreatine.

¹ To whom correspondence should be addressed (e-mail benio@mol.uj.edu.pl).

Table 1 Simulated $t_{1/2}$ values of $\dot{V}O_2$ kinetics in different cases (training statuses)

Training status	$t_{1/2}$ (s)
No parallel activation	83
Untrained muscles (low parallel activation)	49
Trained muscles	
Increase in mitochondrial proteins by 50 %	38
Decrease in the K_m value of mitochondria for ADP by 30 %	43
Increase in mitochondrial proteins by 50 % plus decrease in the K_m value of mitochondria for ADP by 30 %	29
Intensification of parallel activation	24

variables. The set of differential equations is integrated numerically. In each iteration step, new values of rates, concentrations and other parameters are calculated on the basis of the corresponding values from the previous step. The Gear procedure was used for numerical integration and the simulation programs were written in the FORTRAN programming language.

Computer simulations performed during the present study assumed that oxygen delivery by blood at the onset of exercise does not limit oxygen consumption [19–21]. The simulations were made for a moderate exercise intensity, causing a 10-fold increase in oxygen consumption compared with that during rest. It was assumed that ATP supply block in untrained muscles is directly activated $n^{0.2}$ times in parallel with an n -fold activation of ATP usage during muscle contraction (low parallel activation), to obtain $t_{1/2} = 49$ s in untrained muscles, which fits within the range of experimental results reported previously concerning human locomotory muscles [1–5,22]. In subsequent simulations, aimed to reproduce the effect of different possible changes due to muscle training, the activity of (all the complexes of) oxidative phosphorylation was increased by 50 % (which corresponds to the maximum training-induced increase in mitochondria/mitochondrial proteins in humans [9–12]), the phenomenological K_m value of oxidative phosphorylation for ADP was decreased by approx. 30 % [14] or the magnitude of direct activation of oxidative phosphorylation was increased to $n^{0.4}$, to obtain $t_{1/2} = 24$ s in well-trained muscles (shortening of $t_{1/2}$ by 50 % in human locomotor muscles in relation to untrained muscles) [1–5,22]. A similar decrease in the $t_{1/2}$ value can be obtained within the model by increasing mitochondrial volume/mitochondrial proteins by approx. 120 % (2.2 times) or by decreasing the phenomenological K_m value by approx. 70 %. The activation of a given enzyme/process was equivalent to an appropriate increase in the rate constant/maximum velocity of this enzyme/process.

THEORETICAL RESULTS AND DISCUSSION

The simulated $t_{1/2}$ values of $\dot{V}O_2$ kinetics in different cases (training statuses) are presented in Table 1. Experimentally measured training-induced increase in mitochondria/mitochondrial proteins and decrease in the K_m value of oxidative phosphorylation for ADP cannot, by themselves, decrease $t_{1/2}$ to the value of 20–30 s, encountered in well-trained human subjects [1–5,22]. On the other hand, both intensified parallel activation and concomitant increase in the amount of mitochondrial proteins and decrease in K_m value for ADP are able to decrease $t_{1/2}$ to below 30 s. Therefore, to distinguish between different mechanisms enumerated above, it is necessary to compare their impact on the values of $\dot{V}O_2$ and [ADP] during rest-to-work transition.

Figure 1 Simulated steady-state relationship between $\dot{V}O_2$ and free ADP concentration

The simulations are made for untrained muscle and for three cases of trained muscle with different kinds of changes taking place during training. $\dot{V}O_2$ is standardized to 1 for the resting state of untrained muscles.

Figure 1 presents the simulated steady-state relationship between $\dot{V}O_2$ and [ADP] at rest and work of different intensities for four cases: (1) untrained muscles with low parallel activation, (2) trained muscles with a 1.5-fold increase in mitochondrial volume/mitochondrial proteins, (3) trained muscles with a 30 % decrease in K_m value of oxidative phosphorylation for ADP and (4) trained muscles with intensified parallel activation. The most important prediction resulting from Figure 1 is that neither an increase in mitochondrial proteins nor a decrease in K_m value of mitochondria for ADP changes the relative 'phenomenological sensitivity' of oxidative phosphorylation to [ADP], whereas intensified parallel activation increases this sensitivity significantly. Increase in the absolute slope of the $\dot{V}O_2$ /[ADP] relationship, observed for all the three considered mechanisms of the training-induced acceleration of $\dot{V}O_2$ kinetics, is not equivalent to increase in the discussed sensitivity. For instance, increase in the maximum reaction rate V_{max} , which has nothing to do with sensitivity, would bring about the same effect; on the other hand, decrease in K_m value for ADP causes a decrease in resting [ADP] and so the relative proportion of these parameters remains essentially unchanged and the relative 'sensitivity' is unchanged as well. The relative phenomenological sensitivity of oxygen consumption flux to ADP concentration may be defined as follows:

$$R = \frac{\dot{V}O_2(\text{work})/\dot{V}O_2(\text{rest})}{[\text{ADP}]_{(\text{work})}/[\text{ADP}]_{(\text{rest})}} \quad (1)$$

The ratio of R in trained muscle to R in untrained muscle $R_{\text{trained}}/R_{\text{untrained}}$ is equal to 1 for an increase in mitochondria/mitochondrial proteins, for a decrease in the K_m value of oxidative phosphorylation for ADP and for the combination of these two mechanisms. On the other hand, this ratio is essentially > 1 for intensified parallel activation. Therefore the discussed parameter allows one to decide if the contribution of an increase in parallel activation to the training-induced acceleration of $\dot{V}O_2$ kinetics is significant.

Table 2 presents $R_{\text{trained}}/R_{\text{untrained}}$ ratios extracted from three items of relevant literature. One can see that this ratio is significantly > 1 in all three cases. This strongly suggests that muscle training not

Table 2 The ratio of relative 'phenomenological sensitivity' of $\dot{V}O_2$ to [ADP] in trained muscle to the same sensitivity in untrained muscle, calculated on the basis of three sets of experimental results

Muscle	Training/conditioning	$R_{\text{trained}}/R_{\text{untrained}}$	Reference
Latissimus dorsi in dog	Chronic electrical stimulation	3.1	[6]
Hind-limb muscles in rat	Treadmill running training	1.6	[7]
	Exercise training	1.7*	[8]

* For submaximal exercise.

only causes an increase in mitochondria/mitochondrial proteins but also intensifies parallel activation. In fact, without parallel activation, it would be very difficult to explain the fact that a 30-fold increase in $\dot{V}O_2$ in trained muscles is accompanied by only a 2-fold increase in [ADP] during rest-to-work transition, which allows excellent homeostasis of the ATP/ADP ratio to be achieved [8]. As to the decrease in the K_m value of oxidative phosphorylation for ADP, the role of this mechanism is not clear, because in some cases the discussed parameter does not change [14] or even increases [23] as a result of training.

Intensification of parallel activation is equivalent to improvement of the regulatory mechanisms in trained skeletal muscles. In intact heart *in situ*, these mechanisms seem to be perfect, because essentially no changes in [ADP], $[P_i]$ and PCr/ATP take place during a 5-fold increase in work performed by heart muscles and oxygen consumption [24,25]. Therefore trained skeletal muscles begin to resemble heart muscles in some respects.

It has been postulated that the mechanistic sensitivity of oxidative phosphorylation in skeletal muscles to [ADP] is very high (at least second-order dependence) [26]. However, this suggestion remains in disagreement with the kinetic properties of isolated muscle mitochondria and skinned fibres, and is not able to explain the relative stability of NADH [27] and proton-motive force Δp [28] during muscle contraction. Additionally, parallel activation of Δp production and consumption was demonstrated directly during the stimulation of respiration in hepatocytes by vasopressin [29]. Results presented in [26] can be satisfactorily reproduced by the simulated relationship between ATP turnover and [ADP] for trained muscles with increased parallel activation, corresponding to the relevant $\dot{V}O_2$ /ADP relationship presented in Figure 1 (theoretical results not shown). In both cases, a more than 20-fold increase in ATP turnover is accompanied by only approx. 4-fold increase in [ADP]. This difference between the ATP turnover/ADP relationship and the $\dot{V}O_2$ /ADP relationship is due to the proton leak (as presented in Figure 3 in [18]).

It should be emphasized that intensified parallel activation not only accelerates $\dot{V}O_2$ kinetics and improves homeostasis of intermediate metabolite concentrations but also increases the maximum ATP production rate by oxidative phosphorylation.

In the present study, the previously developed model of oxidative phosphorylation in skeletal muscles [18] has been used to illustrate the main thesis of this study. However, theoretical results obtained here are of a general nature and do not depend on the detailed properties of this model. Especially, the $R_{\text{trained}}/R_{\text{untrained}}$ ratio is, in principle, > 1 only for intensified parallel activation.

The physical nature of the factor activating, in parallel, different components of the oxidative phosphorylation system during muscle contraction is not clear, but this factor seems to have much to do with calcium ions. It has been proposed that the factor can be found in the frequency of calcium oscillations generated by cells in response to external stimuli (hormones and neural signals), and that this frequency can be integrated over time by some protein

which causes, e.g., phosphorylation of oxidative phosphorylation complexes [30].

In conclusion, we postulate that two main mechanisms contribute to training-induced adaptation of oxidative phosphorylation in skeletal muscles: (1) increase in mitochondria/mitochondrial proteins and (2) intensified parallel activation of ATP supply and ATP usage. In some cases, these mechanisms can be accompanied by a decrease in the K_m value of oxidative phosphorylation in mitochondria for ADP.

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