Factors determining the oxygen consumption rate ($\dot{V}O_2$) on-kinetics in skeletal muscles

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Using a computer model of oxidative phosphorylation developed previously [Korzeniewski and Mazat (1996) *Biochem. J.* **319**, 143–148; Korzeniewski and Zoladz (2001) *Biophys. Chem.* **92**, 17–34], we analyse the effect of several factors on the oxygen-uptake kinetics, especially on the oxygen consumption rate ($\dot{V}O_2$) and half-transition time $t_{1/2}$, at the onset of exercise in skeletal muscles. Computer simulations demonstrate that an increase in the total creatine pool [PCr + Cr] (where Cr stands for creatine and PCr for phosphocreatine) and in glycolytic ATP supply lengthen the half-transition time, whereas increase in mitochondrial content, in parallel activation of ATP supply and ATP usage, in oxygen concentration, in proton leak, in resting energy demand, in resting cytosolic pH and in initial alkalization decrease this parameter. Theoretical studies show that a decrease in the activity of creatine kinase (CK) [displacement of this enzyme from equilibrium during on-transient (rest-to-work transition)] accelerates the first stage of the $\dot{V}O_2$ on-transient, but slows down

the second stage of this transient. It is also demonstrated that a prior exercise terminated a few minutes before the principal exercise shortens the transition time. Finally, it is shown that at a given ATP demand, and under conditions where CK works near the thermodynamic equilibrium, the half-transition time of $\dot{V}O_2$ kinetics is determined by the amount of PCr that has to be transformed into Cr during rest-to-work transition; therefore any factor that diminishes the difference in [PCr] between rest and work at a given energy demand will accelerate the $\dot{V}O_2$ on-kinetics. Our conclusions agree with the general idea formulated originally by Easterby [(1981) *Biochem. J.* **199**, 155–161] that changes in metabolite concentrations determine the transition times between different steady states in metabolic systems.

Key words: muscle exercise, oxidative phosphorylation, oxygen-uptake kinetics, phosphocreatine, regulation of metabolism.

INTRODUCTION

Oxidative phosphorylation is the main source of ATP used to drive skeletal-muscle contraction at low-to-intensive (but not maximal short-term) exercise. The intensity of ATP usage increases several times during on-transient (rest-to-work transition). The rate of ATP consumption must be matched with the rate of ATP production to avoid significant changes in [ATP] (ATP concentration is essentially constant during rest-to-work transition). The transformation of phosphocreatine (PCr) into creatine (Cr) catalysed by CK (creatine kinase) is the main process that resynthesizes ATP from ADP during the first few seconds of exercise, whereas, later, ATP synthesis is gradually taken over by oxidative phosphorylation in mitochondria. Therefore, the oxygen consumption rate ($\dot{V}O_2$) in muscle increases significantly at the onset of muscle contraction. The oxygen-uptake kinetics during the on-transient, especially the value of the characteristic transition time (τ or half-transition time $t_{1/2}$), is important both for the understanding of the regulation of oxidative phosphorylation in skeletal muscles and for estimation of the exercise capacity in humans. $t_{1/2}$ can also characterize the training status of skeletal muscles and the health status of humans [1–6].

In various experimental studies involving different animals (or humans) and various muscles/muscle groups, and conducted under different experimental conditions, a broad range of $t_{1/2}$ values were observed. Generally, the changes in $\dot{V}O_2$ with time at the onset of exercise are characterized by an exponential or near-exponential behaviour [1,2,7–9]. The reasons for such a value

and not any other value of $t_{1/2}$ in a given case and of the (near-) exponential time course of oxygen uptake are still not fully understood.

It has been proposed that both physiological factors (limitations of oxygen supply to muscle cells by blood) and biochemical factors (intrinsic kinetic properties of oxidative phosphorylation in mitochondria) [10] can determine the oxygen-uptake kinetics at the onset of exercise. Previous studies strongly suggest that oxygen delivery by blood is not limiting for muscle respiration at moderate oxygen uptake rates (essentially below the maximal rate of oxygen consumption, $\dot{V}O_{2,max}$) [11,12]. In the present study, we focus on the biochemical factors shaping the oxygen uptake kinetics at the onset of a moderate-intensity exercise.

It was demonstrated previously that an increase in the initial alkalization of muscle cells (caused by the consumption of protons by CK at the onset of exercise), increase in mitochondrial content, decrease in the K_m value of oxidative phosphorylation for [ADP] and increase in the intensity of the parallel activation in the ATP supply–demand system (direct activation of different oxidative phosphorylation complexes in parallel with the activation of ATP usage during muscle contraction [13–15]) accelerate the $\dot{V}O_2$ on-kinetics (shorten the $t_{1/2}$ values for oxygen consumption at the onset of exercise) [6,16]. It was also proposed why the $t_{1/2}$ value is shorter in trained muscles than in untrained muscle and in neurally stimulated oxidative muscle *in situ* than in electrically stimulated perfused glycolytic muscle [6,15]. However, in the cited papers, only a limited set of factors influencing the oxygen uptake on-kinetics was analysed and changes in $\dot{V}O_2$ (and [PCr])

Abbreviations used: Cr, creatine; CK, Cr kinase; on-transient, rest-to-work transition; PCr, phosphocreatine; Δ PCr, the difference between resting [PCr] and working [PCr]; $\dot{V}O_2$, oxygen consumption rate.

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with time at the onset of exercise for different intensities of these factors were not presented. Additionally, even more important, it was not explained how the mentioned factors determine the $\dot{V}O_2$ on-kinetics at the molecular level.

Easterby [17] demonstrated that the time of transition between different steady states is strictly related to the changes in intermediate metabolite concentrations taking place between these steady states. He defined the transition time $\tau_{A \rightarrow B}$ between a steady state A with flux J_A and a steady state B with flux J_B as being linearly proportional to the sum of changes of intermediate metabolite concentrations between these steady states:

$$\tau_{A \rightarrow B} = \sum_{j=1}^n \Delta[I_j]_{ss} / J_B \quad (1)$$

where $[I_j]_{ss}$ is the steady-state concentration of the intermediate metabolite j . Next, the 'temporal analysis' developed by Easterby was integrated with the metabolic control analysis [18–20] and the temporal control coefficient was defined, which expresses the influence of a given enzyme concentration/activity on the transition time in a given metabolic system. It must be emphasized that Easterby's temporal analysis was developed for sequential (linear or branched) metabolic pathways and not for pathways containing metabolite pairs with moiety conservation property (such as $NADH + NAD^+$ or $PCr + Cr$), that only the final steady state flux (J_B) enters into the definition of $\tau_{A \rightarrow B}$ and that each intermediate concentration has the same contribution to the value of $\tau_{A \rightarrow B}$. As it will be shown below, these properties make the temporal analysis of little use for describing the kinetic properties of oxidative phosphorylation in skeletal muscles.

In the present study, the general concept formulated by Easterby that changes in intermediate metabolite concentrations determine transition times is followed, although it is discussed that Easterby's transition time is only distantly related to $t_{1/2}$, which is used to characterize the transitions between the resting and working steady states in the oxidative phosphorylation system in skeletal muscles.

In the present study, performed using the computer model of oxidative phosphorylation developed previously [21,22], the influence of a broad set of different factors on the oxygen uptake kinetics at the onset of exercise is analysed. It is demonstrated that an increase in the intensity of some factors (mitochondrial content, parallel activation of ATP supply and ATP usage, oxygen concentration, proton leak, resting energy demand, resting cytosolic pH) decreases $t_{1/2}$, whereas an increase in the intensity of some other factors (total creatine pool [$PCr + Cr$], glycolytic ATP supply) increases $t_{1/2}$. It is shown that a decrease in the activity of CK accelerates the first stage of the $\dot{V}O_2$ on-transient, but slows down the second stage of this transient (and therefore makes the time course of $\dot{V}O_2$ strongly non-exponential); because such a behaviour contradicts experimental results, the present study decidedly testifies against any significant displacement of CK from thermodynamic equilibrium (in steady state or, especially, during on-transient). It is also demonstrated that a previous exercise, preceding by a few minutes the principal exercise, significantly shortens the on-transient half-transition time of the latter. It is shown that, at the least, a second-order mechanistic dependence of oxidative phosphorylation on [ADP] postulated by some authors gives a $\dot{V}O_2$ on-kinetics that contradicts experimental results. Finally, it is demonstrated that if CK works near equilibrium, the main parameter at the molecular level that determines the half-transition time of the $\dot{V}O_2$ on-kinetics at a given ATP demand is the amount of PCr that has to be transformed into Cr during a rest-to-work transition.

THEORETICAL PROCEDURES

The previously developed computer model of oxidative phosphorylation in intact skeletal muscles [21] was used in the present theoretical studies. The following enzymes/processes/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, etc.), complex I, complex III, complex IV (cytochrome *c* oxidase), proton leak, ATP synthase, ATP/ADP carrier, phosphate carrier, adenylate kinase, CK and ATP usage. The time variations of the metabolite concentrations, which 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, PCr), are expressed in the form of a set of ordinary differential equations. The other (dependent) variable values (other metabolite concentrations, thermodynamic forces, etc.) are calculated from the independent variable values. The set of differential equations is integrated numerically. In each iteration step, new values of rates, concentrations and other parameters are calculated on the basis of the corresponding values from the previous step. The Gear procedure was used for numerical integration and the simulation programs were written in the FORTRAN programming language. A complete description of the model is available on the web site www.mol.uj.edu.pl/staff/benio.

In all the simulations performed in the present study, ATP demand was increased 26 times at the onset of exercise (medium exercise intensity causing approx. an 11-fold increase in $\dot{V}O_2$ at muscle level in relation to rest; the relative increase in $\dot{V}O_2$ is not equivalent to the relative increase in ATP turnover because of proton leak, as explained in [21]). At the same time, the activities (rate constants) of oxidative phosphorylation enzymes were increased in all simulations (apart from the simulation presented in Figure 7) 26^x times, where $x = 0.3$ under 'standard' conditions (see below). The activation of oxidative phosphorylation did not reach its ultimate value instantaneously at the onset of exercise (because in the real system it most probably takes some time to complete this activation, as a result of for example protein phosphorylation); however it increased exponentially, according to the following expression:

$$m = 1 + (26^x - 1)e^{-t/\tau} \quad (2)$$

where m is the multiplicity of the resting complex activity, t the time and τ the characteristic time constant of the increase in the activation of oxidative phosphorylation, $\tau = 3$ s, which is large enough to avoid a small discontinuity in the time course of $\dot{V}O_2$ that would otherwise take place in the case of instant activation (cf. e.g. [15]), and small enough not to disturb the value of $t_{1/2}$ for oxygen uptake at the onset of exercise. In the simulation presented in Figure 5, the activity of oxidative phosphorylation complexes decreased exponentially with time to the rest value after the termination of exercise, according to the following expression (see [15]):

$$m = 1 + (26^{0.3} - 1)e^{t/\tau} \quad (3)$$

where $\tau = 30$ s during recovery after exercise.

In the simulation presented in Figure 7, concerning the effect of the 'high-sensitivity mechanism' (see below) on the $\dot{V}O_2$ on-kinetics, particular oxidative phosphorylation complexes were not directly activated at the onset of exercise. Instead, in this simulation, the sensitivity of oxidative phosphorylation was increased

by introducing, to the kinetic description of all oxidative phosphorylation complexes, the term $(ADP_{te}/ADP_{te(rest)})^{0.59}$, where ADP_{te} is the total (magnesium-bound + magnesium-free) free cytosolic ADP concentration, $ADP_{te(rest)}$ is the resting total free cytosolic ADP concentration and the power coefficient equal to 0.59 was adjusted to cause the same increase in $\dot{V}O_2$ and [ADP] during rest-to-work transition as in the case of the direct stimulation of oxidative phosphorylation complexes 26^{0.3} times (moderate parallel activation), as described above and below. The above procedure has no mechanistic meaning and is only a simple formal way to increase the sensitivity of oxidative phosphorylation to [ADP].

To study the effect of different factors on the on-transient oxygen uptake kinetics, a standard (reference) set of the intensities of the analysed factors has been established and then the intensities of these factors were changed in subsequent computer simulations. This standard (reference) set of factor intensities corresponds to a 'typical' (rather oxidative than glycolytic) mammalian skeletal muscle under 'typical' physiological conditions; generally, the parameter values were the same as in the original model of oxidative phosphorylation in skeletal muscles [21]. The relevant parameters were as follows: 'typical' mitochondrial activity/volume (corresponding to 7 % of the cytosolic volume), medium parallel activation of ATP supply and ATP usage (power coefficient $x = 0.3$), total creatine pool $[PCr + Cr] = 35$ mM, saturated oxygen concentration $[O_2] = 240$ μ M (of course, this is not a physiological oxygen concentration in blood; however, $[O_2]$ has a very small impact on the properties of the oxidative phosphorylation system in a very broad range of concentrations between 240 and 3 μ M, see below and [23]) and proton leak intensity (or, rather, the rate constant of proton leak), resting ATP usage and CK activity, all three as in [21], and resting cytosolic pH = 7.0. In most simulations, it was assumed that cytosolic pH is constant during exercise and that glycolytic pyruvate production exactly matches pyruvate consumption by oxidative phosphorylation (no anaerobic glycolysis involved and ATP synthesis is by aerobic glycolysis). This assumption seems to be quite a good approximation at moderate exercise intensities [24]. The simulation for the standard (reference) set of factor intensities is shown by a thick line in Figures 1–3, 6 and 7.

To test the effect of a change in the intensity of a given factor on the on-transient oxygen uptake kinetics, the parameter corresponding to this intensity within the computer model of oxidative phosphorylation was changed and a simulation of the rest-to-work transition for the new parameter was made. The extent to which the intensities of particular factors were changed is indicated in Table 1 and in Figures 1–3 and 6. These simulations, corresponding to non-standard factor intensities, are shown by thin lines in Figures 1–3 and 6.

THEORETICAL RESULTS AND DISCUSSION

Table 1 presents the simulated $t_{1/2}$ values for the standard (reference) set of analysed factor intensities (first row in Table 1) and for different intensities of particular factors; in each simulation the value of only one (indicated in the appropriate row in Table 1) factor intensity was modified, whereas the 'standard' values of the remaining factors were kept unchanged.

Figure 1(A) presents the simulated influence of the mitochondrial content/activity on the $\dot{V}O_2$ on-kinetics. It can be seen that an increase in the mitochondrial content significantly decreases $t_{1/2}$ values (see also Table 1). At the same time, an increased mitochondrial content slightly increases the oxygen consumption both at rest and during exercise; this is mainly due to an increased

Table 1 Simulated half-transition times $t_{1/2}$ for different factor intensities

mit, mitochondrial content; x , power coefficient of parallel activation; CK, the concentration/activity of creatine kinase.

Factor intensity	$t_{1/2}$ (s)
Standard (reference) conditions: mit $\times 1$, medium parallel activation ($x = 0.3$), $[PCr + Cr] = 35$ μ M, $[O_2] = 240$ μ M, proton leak $\times 1$, resting ATP usage $\times 1$, resting pH = 7.0, CK $\times 1$	30.4
Mit $\times 0.4$	53.9
Mit $\times 0.7$	37.5
Mit $\times 1.6$	22.1
No parallel activation ($x = 0$)	71.5
Low parallel activation ($x = 0.15$)	47.6
High parallel activation ($x = 0.4$)	21.6
$[PCr + Cr] \times 0.5$	15.1
$[PCr + Cr] \times 0.8$	24.4
$[PCr + Cr] \times 2$	38.4
$[O_2] = 100$ μ M	30.6
$[O_2] = 30$ μ M	31.8
$[O_2] = 10$ μ M	33.9
$[O_2] = 3$ μ M	39.5
Proton leak $\times 0.5$	30.6
Proton leak $\times 2$	29.8
Resting ATP usage $\times 0.5$	35.1
Resting ATP usage $\times 2$	22.8
Resting pH = 6.9	33.3
Resting pH = 7.1	26.7
CK $\times 0.1$	7.5
CK $\times 0.3$	23.4
CK $\times 10$	31.9

proton leak at high phosphorylation potential (and proton motive force Δp) (results not shown).

Figure 1(B) presents the simulated effect of the mitochondrial content/activity on the changes of [PCr] with time during rest-to-work transition. One can see that the high oxidative phosphorylation activity increases the resting PCr level (and thus the PCr/Cr ratio); this is a result of an increase in the ATP/ADP ratio (PCr/Cr ratio reflects the ATP/ADP ratio through the CK equilibrium). At the onset of exercise, PCr takes a much longer time to reach its active (working) steady-state level at low mitochondrial content than at high mitochondrial content. This is a direct consequence of the fact that the difference between resting [PCr] and working [PCr] is much greater in the former case than in the latter.

The $\dot{V}O_2$ on-kinetics (including $t_{1/2}$) is mainly determined by the kinetics of the increase in [ADP] (and, to a lesser extent, in $[P_i]$; both compounds activate oxidative phosphorylation) from resting [ADP] value to active (working) [ADP] value. Changes in free [ADP] are reflected by changes in Cr/PCr ([ATP] is essentially constant during a rest-to-work transition in skeletal muscles because of a very high ATP/ADP ratio). At high resting PCr concentrations, small changes in [PCr] cause large relative changes in Cr (and in PCr/Cr) and therefore large changes in [ADP], whereas at low resting PCr concentrations much greater changes in [PCr] are needed to cause the same large relative changes in PCr/Cr and [ADP] (resting PCr/Cr ratio > 1 , and in a typical case has a value approx. 3–4). Since [Cr] is approx. three orders of magnitude higher than [ADP], changes in [Cr] or [PCr] ($[Cr] + [PCr] = \text{const.}$) determine the time of transition from resting [ADP] to working [ADP] (cf. [17]). At a given level of ATP usage, larger changes in [PCr] need more time to be completed. Therefore, the amount of PCr (or Cr) that has to be transformed into Cr (or PCr) during a rest-to-work transition determines the

Figure 1 Simulated changes of $\dot{V}O_2$ (A) and [PCr] (B) as a function of time at the onset of exercise for different mitochondrial contents/activities

$\text{Mit} \times n$ represents the mitochondrial content/activity in a given simulation and is n times greater than the 'standard' mitochondrial content/activity. $\dot{V}O_2$ is standardized to unity for resting state under 'standard' conditions (factor intensities).

half-transition time of [ADP] and thus the half-transition time of $\dot{V}O_2$. (However, one must remember that the dependence of $\dot{V}O_2$ on [ADP] is not linear but hyperbolic, and that oxidative phosphorylation is less saturated with ADP at high resting [PCr] than at low resting [PCr]). This is the reason why an increase in mitochondrial activity that decreases ΔPCr (the difference between resting [PCr] and working [PCr]) accelerates the oxygen uptake kinetics at the onset of exercise.

Generally, time-dependent changes in both $\dot{V}O_2$ and [PCr] have a near-exponential shape and are characterized by almost the same value of $t_{1/2}$ for each value of mitochondrial content/activity. This near-exponential shape is determined by the near-linear relationship between ATP (and PCr) production and [Cr] [21]. Therefore, the rate of changes in PCr concentration can be approximately expressed as follows (assuming that CK works near thermodynamic equilibrium):

$$d\text{PCr}/dt = k \cdot (\text{Cr} - \text{Cr}_{\text{work}}) \quad (4)$$

or in an equivalent way,

$$d\text{PCr}/dt = k \cdot (\text{PCr}_{\text{work}} - \text{PCr}) \quad (5)$$

Figure 2 Simulated changes of $\dot{V}O_2$ (A) and [PCr] (B) as a function of time at the onset of exercise for different parallel activation intensities

$x = 0.4$, high parallel activation; $x = 0.3$, medium parallel activation; $x = 0.15$, low parallel activation; $x = 0$, no parallel activation. $\dot{V}O_2$ is standardized to unity for resting state under 'standard' conditions (factor intensities).

where k is an appropriate rate constant, Cr_{work} and PCr_{work} are active (working) steady-state concentrations of Cr and PCr determined by the balance between ATP (and PCr) consumption and ATP (and PCr) production. As is well known, integration of eqns (4) and (5) provides an exponential function.

The only significant deviation from the exponential increase in time of $\dot{V}O_2$ at the onset of exercise can be seen during the first 3 s of the exercise, where $\dot{V}O_2$ increases very quickly due to the direct activation of oxidative phosphorylation. In measurements of the pulmonary oxygen uptake, this effect is most probably 'masked' by the cardiodynamic component of the on-transient $\dot{V}O_2$ kinetics.

Figure 2 presents the simulated effect of the intensity of parallel activation in the ATP supply–usage system (direct activation of different oxidative phosphorylation complexes during muscle contraction) postulated previously [13–15] on the on-transient of $\dot{V}O_2$ (Figure 2A) and [PCr] (Figure 2B). As mentioned in the Theoretical procedures section, parallel activation is expressed within the computer model of oxidative phosphorylation used in the present study due to the fact that all oxidative phosphorylation enzymes are activated n^x times in parallel with an n -fold activation of ATP usage during muscle contraction; the value of x is a measure of the intensity of parallel activation. As one can easily see in Figure 2 (also compare with Table 1), increase in parallel

activation accelerates the transition from resting steady state to working steady state (decreases $t_{1/2}$) in both cases (for $\dot{V}O_2$ and [PCr]). However, unlike the case of increase in mitochondrial content, increase in parallel activation does not affect the resting level of $\dot{V}O_2$ and [PCr], because parallel activation takes place only during muscle contraction.

Increase in parallel activation exerts its effect on the on-transient kinetics and $t_{1/2}$ for $\dot{V}O_2$ through the change in the difference between resting [PCr] and working [PCr] (Δ PCr), similar to the results of an increase in mitochondrial content; also here, the half-transition times for $\dot{V}O_2$ and for [PCr] are essentially the same for every parallel activation intensity. However, the reason for the change in Δ PCr is different in both cases. Increase in parallel activation results in an additional direct activation of oxidative phosphorylation during muscle contraction and, therefore, a smaller increase in [ADP], and hence in the Cr/PCr ratio, is needed to cause the same increase in $\dot{V}O_2$ during rest-to-work transition as before an intensification of parallel activation. Shapes of the curves presented in Figure 2 are near-exponential at all parallel activation intensities for the reasons discussed above.

The simulated effect of the size of (PCr + Cr) on the half-transition time of the oxygen uptake kinetics is presented in Table 1. An increase in [PCr + Cr] slows down the $\dot{V}O_2$ kinetics and lengthens the half-transition time. As mentioned previously, the main factor determining the on-transient $\dot{V}O_2$ kinetics and $t_{1/2}$ is the difference (Δ PCr) between resting [PCr] and working [PCr]. Of course, an increase in the total creatine pool increases also the resting PCr concentration ([PCr]/[Cr] remains essentially unchanged; results not shown). However, in this case, an increase in resting [PCr] is accompanied by an increase in the half-transition time for oxygen uptake kinetics. Therefore, the 'true' parameter determining $t_{1/2}$ is Δ PCr and not resting [PCr] (see the discussion below). A similar influence of the total creatine pool on the half-transition time was found experimentally and predicted from a simple model involving a linear dependence of ATP production by oxidative phosphorylation on [Cr] [25].

As can be seen in Table 1, $[O_2]$ has only a very small impact on $t_{1/2}$ over a very broad range from 240 μ M (saturated oxygen concentration) to 3 μ M. The model predicts the K_m value of oxidative phosphorylation for extracellular $[O_2]$ to be approx. 0.8 μ M, in accordance with the experimental values reported for isolated cells. If extracellular oxygen concentration decreases to values close to, or lower than, the K_m value, it will affect both the oxygen consumption and $t_{1/2}$ values. However, such a situation can take place only in a very severe hypoxia in skeletal muscles.

It is possible that the oxygen concentration inside the cells, near mitochondria, is lower than the extracellular oxygen concentration because of diffusion gradients. However, the K_m value for $[O_2]$ of oxidative phosphorylation in isolated mitochondria is much lower than that in intact cells; it is approx. 0.1 μ M [26]. Therefore, oxidative phosphorylation is (almost) saturated with oxygen under most conditions. Generally, $[O_2]$ does not seem to affect significantly the oxygen uptake kinetics at oxygen uptake rates much lower than $\dot{V}O_{2,max}$ [11,12].

Figure 3 presents the simulated effect of the intensity (or, more strictly, of the rate constant) of the proton leak through the inner mitochondrial membrane on the $\dot{V}O_2$ on-kinetics. It can be seen that an increase in proton leak increases the resting and working oxygen consumption, and shortens $t_{1/2}$ (see also Table 1). The proton leak intensity may be affected by, for example, training-induced decrease in the amount of the uncoupling protein 3 (UCP3) in skeletal muscles [27]. A similar effect (i.e. decrease in $t_{1/2}$ and increase in the resting and working oxygen consumption) is brought about by an increase in resting ATP usage (by 'basic' processes keeping the cell alive; results not shown; see Table 1).

Figure 3 Simulated changes of $\dot{V}O_2$ as a function of time at the onset of exercise for different intensities of proton leak

Proton leak $\times n$ represents the rate constant of proton leak in a given simulation and is n times greater than the 'standard' rate constant of proton leak. $\dot{V}O_2$ is standardized to unity for resting state under 'standard' conditions (factor intensities).

Figure 4 Simulated correlation between $t_{1/2}$ of $\dot{V}O_2$ on-kinetics and the difference in PCr concentration between rest and work (Δ PCr)

The presented relationship is generated by different intensities of six factors influencing $t_{1/2}$: $\square$, [PCr + Cr]; $\blacktriangledown$, mitochondrial content; $\triangle$, parallel activation intensity; $\bullet$, oxygen concentration; $\blacktriangle$, proton leak; $\blacksquare$, resting ATP usage. The reference point corresponds to 'standard' conditions assumed in the model.

In both cases, the $\dot{V}O_2$ kinetics is accelerated by the fact that the studied factors lower the resting PCr concentration, whereas the working steady-state [PCr] is less affected; hence Δ PCr is decreased (results not shown).

Increase in the resting pH decreases $t_{1/2}$ for the $\dot{V}O_2$ on-kinetics (see Table 1) because a decrease in proton concentration shifts the CK equilibrium towards a higher resting PCr concentration, which causes a smaller amount of PCr to get transformed into Cr during rest-to-work transition, as discussed above for an increase in mitochondrial content/activity.

Figure 4 presents the simulated relationship between $t_{1/2}$ for the $\dot{V}O_2$ on-kinetics and Δ PCr (the difference between resting [PCr] and working [PCr]). Variations in both parameter values were caused by setting different intensities of various factors influencing the oxygen uptake kinetics at the onset of exercise, as indicated in the legend of Figure 4. The simulations were

performed for the same intensity of ATP usage, with CK working near thermodynamic equilibrium and with no ATP production by anaerobic glycolysis. It can be seen that the relationship between $t_{1/2}$ and ΔPCr is generally linear and passes through the origin of the co-ordinate system. This fact illustrates well that ΔPCr is the 'true' main 'primary' determinant of $t_{1/2}$ under these conditions. It has been proposed that $t_{1/2}$ is proportional to the resting PCr concentration [28]. It is true that resting [PCr] is negatively correlated with ΔPCr (see above) at a constant total Cr pool. However, the relationship between $t_{1/2}$ and resting [PCr] is not linear (not shown). In addition, it is important to note that it is possible, by manipulating with [PCr + Cr] and with for example mitochondrial content, to obtain two different values of ΔPCr and $t_{1/2}$ at the same resting PCr concentration. Therefore, there is no unique relationship between $t_{1/2}$ and resting [PCr].

The definition of the transition time proposed by Easterby ([17] and eqn 1) also predicts that the transition time is linearly proportional to changes in metabolite concentrations. However, the absolute values of $\tau_{A \rightarrow B}$ calculated on the basis of this definition would be much smaller than the values of $t_{1/2}$ presented in Figure 4 because eqn (1) takes into account the final steady-state flux J_B (and not the average flux during transition) and because the 'temporal analysis' does not take into account the moiety conservation property, as discussed in detail below. Generally, the linearity seen in Figure 4 is by no means a direct consequence of Easterby's temporal analysis; in many cases, for example if CK is significantly displaced from thermodynamic equilibrium, the relationship between $t_{1/2}$ and changes in metabolite concentrations will not be linear. The linear relationship simulated in the present study is a consequence of the fact that ATP usage is kept constant and that PCr changes are always near-exponential in form when creatine kinase works near thermodynamic equilibrium. However, the discussed kinetic behaviour of the system is not trivial and obviously true if one takes into account the fact that (i) the dependence of $\dot{V}\text{O}_2$ on [ADP] is hyperbolic, (ii) P_i also activates oxidative phosphorylation to some extent, (iii) the strongly non-ohmic proton leak contributes to the $\dot{V}\text{O}_2/\text{ADP}$ relationship and (iv) a parallel activation in the ATP supply-demand system takes place.

Even though the relationship presented in Figure 4 is generally linear, there are some slight deviations from an ideal linear relationship. They are caused by (1) the fact that not only ADP, but also P_i activates, although to a smaller extent, the oxidative phosphorylation system in mitochondria (therefore, the reasoning presented above on the basis of the equilibrium between ATP/ADP and PCr/Cr is only approximately valid); (2) small displacement of CK from equilibrium during the transient state; and (3) contribution of the proton leak to the oxygen consumption. Therefore, ΔPCr is the main kinetic parameter determining $t_{1/2}$ under the considered conditions, but there are also some additional, minor parameters that matter.

A few minutes after termination of an exercise, if [PCr] did not return to its resting level and a new exercise is started, ΔPCr for this principal exercise will of course be smaller than for the previous exercise and therefore $t_{1/2}$ also will be smaller. This is illustrated by the simulation presented in Figure 5, where the principal exercise is started 6 min after the termination of the previous exercise and PCr did not have enough time to return to its resting value (this theoretical prediction is confirmed by experimental studies [24,29]). Such a lowering of $t_{1/2}$ value in the principal exercise was encountered in experimental studies [30,31] and we postulate that a slow return of [PCr] to the resting value may be one of the reasons for this phenomenon.

The above simulations were performed under the assumption that (i) CK works near thermodynamic equilibrium, (ii) this

Figure 5 Simulated changes of $\dot{V}\text{O}_2$ and [PCr] as a function of time at the onset of exercise for two exercises separated by 6 min of recovery

$\dot{V}\text{O}_2$ is standardized to unity for resting state.

Figure 6 Simulated changes of $\dot{V}\text{O}_2$ as a function of time at the onset of exercise for different activities of CK

$\text{CK} \times n$ represent the rate constants of CK in a given simulation and are n times greater than the 'standard' rate constants of CK. $\dot{V}\text{O}_2$ is standardized to unity for resting state at 'standard' conditions (factor intensities).

equilibrium is not shifted during on-transient, (iii) ATP usage is identical in each case and (iv) there is no ATP production by anaerobic glycolysis. However, it is interesting to check the consequences of one of these assumptions not being fulfilled.

Figure 6 presents the simulated effect of the activity of CK on the $\dot{V}\text{O}_2$ on-kinetics. Decrease in the CK activity below some critical value causes the reaction catalysed by this enzyme to be slow and therefore displaced from equilibrium during the transient state at the onset of exercise. Therefore, Figure 6 shows in fact the effect of the displacement of CK from equilibrium on the oxygen uptake kinetics at the onset of exercise. It can be seen that a decrease in CK activity strongly accelerates the first phase of $\dot{V}\text{O}_2$ on-transient, which is associated with a very small value of $t_{1/2}$ (see Table 1), and at the same time it essentially slows down the second phase of $\dot{V}\text{O}_2$ on-transient. As a result, the time course of the oxygen consumption at the onset of exercise becomes strongly non-exponential. Such a behaviour was never observed in real systems. This fact constitutes a strong argument against the significant displacement of CK from equilibrium postulated by some authors (see e.g. [32]).

The kinetic behaviour described above is caused by the fact that CK works too slowly to cause a quick resynthesis of ATP from

ADP at the onset of muscle contraction causing an increased ATP consumption. Consequently, [ADP] increases very quickly above the value that causes the half-maximal increase in $\dot{V}O_2$ (at a given energy demand). Only then are the equilibrium values of ATP/ADP and PCr/Cr slowly approached.

In our previous paper [16], we have shown that the initial alkalization of the muscle cell at the onset of exercise caused by proton consumption by CK shortens the half-transition time of the $\dot{V}O_2$ uptake kinetics at the onset of the exercise. This effect is caused by a shift in the CK equilibrium brought about by changing the proton concentration.

Dependence of the value of $t_{1/2}$ on the exercise intensity depends essentially on the kinetic description of parallel activation (results not shown). The description we use (n^*) is certainly only an approximation of the reality and therefore we did not study in detail the effect of intensity on the half-transition time of the oxygen uptake kinetics at the onset of exercise.

ATP production by anaerobic glycolysis lengthens $t_{1/2}$ (results not shown). We have at our disposal only a preliminary kinetic description of ATP and H^+ production by anaerobic glycolysis, but several different kinetic descriptions tested by us gave the same general, semi-quantitative result. In fact, this effect is logical, because any additional ATP supply slows down the increase in ADP concentration at the onset of exercise to the level that causes half-maximal increase in $\dot{V}O_2$ (at a given ATP demand).

In our previous papers [6,13–15], we have argued that the parallel-activation mechanism is responsible for the large increase in $\dot{V}O_2$ accompanied by only moderate changes in [ADP] and other intermediate metabolite concentrations during rest-to-work transition in skeletal muscles. However, Jeneson et al. [33] proposed an alternative explanation of this phenomenon: at least a second-order mechanistic dependence of oxidative phosphorylation on [ADP]. We argued [13–15] that the latter mechanism, which can be called the ‘high-sensitivity mechanism’, is not able to account for several other kinetic properties of oxidative phosphorylation in skeletal muscles. To continue the comparison between both mechanisms, we simulated the $\dot{V}O_2$ on-kinetics for the high-sensitivity mechanism. (As explained in the Theoretical procedures section, we increased the mechanistic sensitivity of oxidative phosphorylation in our model to an extent that gives exactly the same increase in $\dot{V}O_2$ and [ADP] during rest-to-work transition as the parallel activation mechanism.) The results of this simulation are presented in Figure 7. It can be easily seen that the high-sensitivity mechanism predicts $\dot{V}O_2$ on-kinetics to be slightly sigmoidal and decidedly non-exponential. Since such kinetics are not observed in experimental studies, the presented simulation constitutes a subsequent argument against the high-sensitivity mechanism.

As mentioned in the Introduction section, the general idea that the time of transition between different steady states in metabolic systems is determined by changes in intermediate metabolite concentrations between these steady states comes originally from Easterby [17]. However, for several reasons, the transition time $\tau_{A \rightarrow B}$ (eqn 1) defined by this author is only distantly related to the half-transition time $t_{1/2}$ used in the present study and it is difficult to apply $\tau_{A \rightarrow B}$ to the oxidative phosphorylation system in skeletal muscles.

First, Easterby’s temporal analysis was developed for sequential metabolic pathways (it was also applied to branched metabolic pathways [20]). However, it is not clear how it relates to biochemical systems that contain metabolite pairs (such as NADH + NAD⁺ or PCr + Cr) characterized by the moiety conservation property according to which the sum of metabolite concentrations in a given pair is constant. During transitions between different steady states, concentrations of metabolites in such pairs change

Figure 7 Simulated changes of $\dot{V}O_2$ as a function of time at the onset of exercise for different mechanisms of the regulation of oxidative phosphorylation

$\dot{V}O_2$ is standardized to unity for resting state at ‘standard’ conditions (factor intensities).

in opposite directions and therefore the overall change is exactly zero. This would imply, owing to Easterby’s definition (eqn 1), that for example changes in the PCr/Cr ratio have no influence on the transition time, which is obviously nonsensical. Furthermore, the size of the total creatine pool [PCr + Cr] should not influence the transition time, which is in contradiction with the simulations presented in Table 1. The oxidative phosphorylation system which is described explicitly within our model is composed of exclusively metabolite pairs (or groups) with the moiety conservation property. These groups comprise NADH + NAD⁺ pair, UQH₂ + UQ pair, cyt.*c*²⁺ + cyt.*c*³⁺ pair, adenine nucleotide pool, total creatine pool and the total phosphate pool. As a consequence, the sum of metabolite concentrations changes little between different steady states, or not at all, if the redistribution of inorganic phosphate between cytosol and mitochondria is neglected. On the other hand, it is evident that changes in for example PCr/Cr have a very strong impact on the half-transition time $t_{1/2}$. It is possible that Easterby’s temporal analysis can be modified to overcome the above problem, for instance, by taking into account only one metabolite in a pair. However, we are not aware of any such modification available in the literature.

Secondly, the value of $\tau_{A \rightarrow B}$ may be negative if metabolite concentrations decrease during transition between steady states, whereas $t_{1/2}$ is always positive. This may be only a matter of convention, but $\tau_{A \rightarrow B}$ and $t_{1/2}$ differ in this respect.

Thirdly, eqn (1) does not take into account the fact that concentrations of different metabolites may have a different influence on fluxes and thus on transition times related to these fluxes. For instance, [Cr] and [P_i] change to a comparable extent during transitions between different steady states in skeletal muscles, but [Cr] has a much stronger effect (via [ADP]) on the oxygen consumption flux than [P_i] and therefore it also has a stronger impact on the value of the half-transition time.

Finally, the greatest weakness, at least in our opinion, of Easterby’s transition time is the fact that only the flux in the final steady state (J_B) enters into the definition of this time (see eqn 1). The relevant problem can be very well seen when we consider the rest-to-work transition and the reverse work-to-rest transition in skeletal muscles. Of course, the sum of the changes of metabolite concentrations:

$$\sum_{j=1}^n \Delta[I_j]_{ss}$$

will be identical for both transitions (although it will have the opposite sign). However, when $\dot{V}O_2$ is, say, 20 times greater during work than in rest, J_B will also be 20 times greater during rest-to-work transition than during work-to-rest transition. As a consequence, the transition time $\tau_{A \rightarrow B}$ during on-transient would be 20 times smaller than the value during off-transient. On the other hand, $t_{1/2}$ is either identical in both cases or differs by up to about 1.5 times (see e.g. [15] for a discussion). Generally, Easterby's temporal analysis underestimates transition times when the flux increases, and overestimates transition times when the flux decreases during transitions between different steady states. Therefore it works best if the changes in the flux are small. To overcome this problem, in our opinion, eqn (1) should be modified by replacing the final flux J_B by the average transition flux J_{AB} (which is somewhere between the initial flux J_A and the final flux J_B).

Generally, although we fully accept the general idea formulated by Easterby that changes in metabolite concentrations determine transition times in metabolic systems, we think that his definition of the transition time $\tau_{A \rightarrow B}$ is not very useful for the analysis and understanding of the transition between different steady states of the oxygen consumption flux in skeletal muscles.

In summary, we have identified the main parameter, ΔPCr , that determines $t_{1/2}$ of oxygen uptake kinetics at the onset of skeletal-muscle exercise for a given ATP usage and under the assumption that CK works near thermodynamic equilibrium. We show that any significant displacement of CK from equilibrium predicts kinetic behaviour of the system (oxidative phosphorylation in intact skeletal muscles), contradicting experimental results. Finally, we demonstrate that several factors, such as mitochondrial content, intensity of parallel activation in the ATP supply-demand system, total $[PCr + Cr]$, oxygen concentration, resting pH, proton leak and resting ATP consumption may affect, through changes in ΔPCr , the $\dot{V}O_2$ on-kinetics and $t_{1/2}$ for oxygen uptake at the onset of exercise.

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