Slow $\dot{V}O_2$ off-kinetics in skeletal muscle is associated with fast PCr off-kinetics—and inversely

Bernard Korzeniewski and Jerzy A. Zoladz

Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland; and Department of Muscle Physiology, Chair of Physiology and Biochemistry, Faculty of Rehabilitation, University School of Physical Education, Kraków, Poland

Submitted 15 April 2013; accepted in final form 17 June 2013

Korzeniewski B, Zoladz JA. Slow $\dot{V}O_2$ off-kinetics in skeletal muscle is associated with fast PCr off-kinetics—and inversely. J Appl Physiol 115: 605–612, 2013. First published June 20, 2013; doi:10.1152/japplphysiol.00469.2013.—The computer model of the bioenergetic system in skeletal muscle, developed previously, was used to study the effect of the characteristic decay time of the parallel activation of oxidative phosphorylation [$\tau_{\text{OFF}}$] during muscle recovery on the muscle oxygen consumption rate ($\dot{V}O_2$) and phosphocreatine (PCr) work-to-rest transition (off)-kinetics and on the relationship between the VO$_2$ and PCr rest-to-work transition (on)- and off-kinetics in moderate and heavy exercise. An increase in $\tau_{\text{OFF}}$ slows down the initial phase of the muscle VO$_2$ off-kinetics and accelerates the PCr off-kinetics. As a result, the relationship between the initial phase of the VO$_2$ off-kinetics (lasting approximately 3-60 s in computer simulations) and the PCr off-kinetics is inverse: the slower the former, the faster the latter. A faster initial phase of the VO$_2$ off-kinetics is associated with a slower late phase of the VO$_2$ off-kinetics, and as a result, the integral of VO$_2$ above baseline during recovery, representing the oxygen debt, is identical in all cases [values of $\tau_{\text{OFF}}$] for a given PCr decrease. Depending on $\tau_{\text{OFF}}$, the muscle VO$_2$ on-kinetics was either equally fast or slower than the VO$_2$ off-kinetics in moderate exercise and always slower in heavy exercise. PCr on-kinetics was always faster than PCr off-kinetics. This study clearly demonstrates that $\tau_{\text{OFF}}$ has a pronounced impact on the mutual relations between the muscle VO$_2$ and PCr on- and off-kinetics.

VO$_2$ off-kinetics; VO$_2$ on-kinetics; skeletal muscle; parallel activation; computer model

It has been shown that the pulmonary oxygen consumption rate ($\dot{V}O_2$) and phosphocreatine (PCr) rest-to-work transition (on)- and work-to-rest transition (off)-kinetics is exponential for both moderate and heavy work intensity [see, e.g., Rossiter and coworkers (27, 28)]. It has been demonstrated that the primary time constants ($\tau_p$) of the muscle and pulmonary VO$_2$ on-kinetics are similar for moderate work intensity in humans (4). Little, however, is known about the relationship between VO$_2$ on- and off-kinetics at the level of working muscle during exercise. It has been shown in one experimental set that the muscle VO$_2$ on- and off-kinetics is symmetrical for low-intensity contraction (muscle VO$_2$ during work, only 4.5 times higher than at rest) (2). Even less is known about the relationship between muscle VO$_2$ and PCr on- and off-kinetics in the working muscles. It has been shown that the $\tau_p$ of the fundamental component for the pulmonary VO$_2$ on-kinetics and the fundamental component for the PCr on-kinetics in human quadriceps muscle during voluntary exercise are very similar, both during the exercise of moderate and heavy intensities (28). Similarly, the $\tau_p$ of the PCr off-transition was closely related to the $\tau_p$ of pulmonary VO$_2$ off-transition, after exercise of both moderate and heavy intensities (28). Moreover, a pronounced asymmetry of PCr and pulmonary VO$_2$ kinetics between the on- and off-transients of moderate and high-intensity exercise was reported (faster on- than off-kinetics) (28). The recent study by Wüst and coworkers (34) showed that in electrically stimulated dog gastrocnemius muscle, $\tau_p$ of the VO$_2$ on-kinetics was much shorter than the $\tau_p$ of the PCr on-kinetics. These results suggest that the close relationship between the PCr on-kinetics and the pulmonary VO$_2$ on-kinetics reported previously (28) might actually be different from the muscle VO$_2$ and PCr on-kinetics relationship in the working muscle, at least in some experimental systems and conditions (e.g., heavy exercise).

It was demonstrated in our previous in silico studies (19) that an increase in the characteristic decay time of the parallel activation of oxidative phosphorylation [$\tau_{\text{OFF}}$] (12–14) during muscle recovery accelerates the rate of PCr resynthesis and therefore, shortens its half-transition time ($\tau_{1/2\text{off}}$). This theoretical result was confirmed by Kemp (9). It was also predicted that a long-enough $\tau_{\text{OFF}}$ leads to an appearance of the PCr recovery overshoot (13, 19).

To our knowledge, no studies, either experimental or theoretical, have been carried out to analyze the impact of the rate of the decay of parallel activation of oxidative phosphorylation on the muscle VO$_2$ off-kinetics and the relationship between the muscle VO$_2$ and PCr off-kinetics. In the present article, we hypothesize that the relationship between the initial phase of the VO$_2$ off-kinetics and the PCr off-kinetics is inverse: the slower the initial phase of the VO$_2$ off-kinetics, the faster the PCr off-kinetics—and inversely. We also test the supposition that the $\tau_{\text{OFF}}$ during muscle recovery has a pronounced impact on the mutual relations between the muscle VO$_2$ and PCr on- and off-kinetics.

THEORETICAL METHODS

Computer model. The theoretical model of skeletal muscle cell bioenergetics, including anaerobic glycolysis, developed by Korzeniewski and Liguzinski (15), based on the earlier model by Korzeniewski and Zoladz (16), was used in the present study. This model comprises oxidative phosphorylation, anaerobic glycolysis, creatine kinase (CK), ATP use, NADH supply, and proton efflux.

The model has been broadly validated by comparing its predictions with experimental data and has been used for numerous theoretical studies (10–20, 36). The complete model description of the skeletal muscle bioenergetic system, including anaerobic glycolysis, is given...
The simulations were made for two exercise intensities: moderate exercise and heavy exercise. We assumed that the activity (rate constant) of ATP use (hydrolysis) is elevated 30 times during transition from rest to moderate exercise and 80 times during transition from rest to heavy exercise. This gave the value of muscle V̇O₂ equal to ~3.7 mM/min during moderate exercise and to ~9.2 mM/min during heavy exercise. During single muscle exercise, the muscle V̇O₂max equals ~15–25 mM/min (1, 25) in human quadriiceps. During whole-body exercise (cycling), the muscle V̇O₂max in young, healthy, physically active men could be as high as 10–13 mM/min (32, 38), however, it is reasonable to assume that some muscles in this group, for instance, quadricepses, work with a much greater intensity than others, and therefore, their V̇O₂max is much higher. In electrically stimulated dog gastrocnemius, but is of the same order of magnitude. It was assumed that there is essentially no anaerobic glycolysis (and consequently, no net H⁺ and lactate production) in moderate exercise [pyruvate and NADH supply exactly equal to NADH use; see, e.g., Zoladz et al. (38)]. During transition to heavy exercise, glycolysis was activated (its rate constant, kGLYC, was elevated) instantly, 80–17 times at the onset of exercise. The need for this direct parallel activation of glycolysis was demonstrated in Korzeniewski and Liguzinski (15).

After termination of exercise, the rate constants of ATP use and glycolysis were decreased instantly to the initial (rest) values. The rate constants of oxidative phosphorylation complexes decreased in the exponential way due to the following equation (13)

\[
m = N^P - (N^P - 1) \cdot e^{-\frac{t}{\tau_{ON}}}
\]

where \( m \) is the current activation (ratio of the current rate constant to the resting rate constant), and \( N \) is the relative activation of ATP use during exercise (30 and 80 for moderate and heavy exercise, respectively); the power coefficient, \( p = 0.4 \), expresses the intensity of parallel activation, \( \tau(ON) \) is the characteristic activation time of the increase of oxidative phosphorylation, and \( t \) is the time after the onset of exercise. \( \tau(ON) \) was equal to 3 s. This value is smaller than 11 s, estimated in Wüst et al. (34) for electrically stimulated dog gastrocnemius, is of the same order of magnitude. It was assumed that there is essentially no anaerobic glycolysis (and consequently, no net H⁺ and lactate production) in moderate exercise [pyruvate and NADH supply exactly equal to NADH use; see, e.g., Zoladz et al. (38)]. During transition to heavy exercise, glycolysis was activated (its rate constant, kGLYC, was elevated) instantly, 80–17 times at the onset of exercise. The need for this direct parallel activation of glycolysis was demonstrated in Korzeniewski and Liguzinski (15).

In the exponential function, \( \tau \) is the time necessary for a given variable value (for instance, V̇O₂ or PCr) to undergo a change equal to 63% (0.63) of the final (complete) change during a given transition. Because in our simulations, the transitions of V̇O₂ and PCr are near exponential (or in some cases, not exponential at all), we use the characteristic transition time \( t_{0.63} \) instead of \( \tau \), as the time necessary to undergo 63% of the final (complete) change to characterize these transitions and to compare computer simulations with experimental results.

### RESULTS

The simulations of on- and off-transient for different \( \tau(ON) \) values for moderate exercise reveal, first of all, that the relationship between the V̇O₂ and PCr off-kinetics is inverse: the faster the former, the slower the latter—and inversely. This is demonstrated in Fig. 1. Changes in chosen variable values during moderate on-transition are presented in Table 1. Muscle

![Fig. 1. Simulated work-to-rest transition (off)-kinetics of oxygen consumption rate (V̇O₂) and phosphocreatine concentration ([PCr]) for different values of the characteristic decay time of the activation of oxidative phosphorylation \( \tau(ON) \) after moderate exercise. A: V̇O₂ rest-to-work transition (on)- and off-kinetics. Inset: enlarged fragment of A. B: [PCr] on- and off-kinetics.](image-url)
\[ \text{VO}_2 \text{ increases 13.0 times, ADP concentration ([ADP]) increases 3.05 times, pH and [ATP] are essentially constant, [PCr] decreases to 75\% of its initial value, and [Pi] increases 3.01 times. An increase in } \tau(\text{OFF}) \text{ from 0 s through 10 s, 30 s, and 100 s to 1,000 s causes an increase in } t_{0.63\text{off}} \text{ for } \text{VO}_2 \text{ from approximately 0 s to 19.6 s and decrease in } t_{0.63\text{off}} \text{ for PCr from 174.4 s to 23 s (see Table 2). At } \tau(\text{OFF}) \text{ of 100 s and 1,000 s, a significant PCr recovery overshoot appears (see Fig. 1). } t_{0.63} \text{ for } \text{VO}_2 \text{ is essentially identical for on- and off-transient (symmetry) at } \tau(\text{OFF}) \text{ of 1,000 s, similar at } \tau(\text{OFF}) \text{ of 100 s, and significantly shorter for off- than for on-transient at other } \tau(\text{OFF}) \text{ values. } t_{0.63} \text{ for PCr is moderately longer for off- than for on-transient at } \tau(\text{OFF}) \text{ of 1,000 s and 100 s and significantly longer at lower values of } \tau(\text{OFF}). \text{ The relation between } t_{0.63\text{off}} \text{ for } \text{VO}_2 \text{ and PCr is near symmetrical: the on-transient for PCr is only slightly shorter than for } \text{VO}_2. \text{ The difference between } t_{0.63\text{off}} \text{ for } \text{VO}_2 \text{ and PCr changes from moderate at } \tau(\text{OFF}) \text{ of 1,000 s and 100 s to huge at } \tau(\text{OFF}) \text{ of 10 s and 0 s (} t_{0.63\text{off}} \text{ for } \text{VO}_2 \text{ is always shorter). This pattern changes quite significantly at heavy exercise. This can be seen in Fig. 2 and Tables 1 and 3. The relationship between the } \text{VO}_2 \text{ and PCr off-kinetics remains inverse. However, changes in fluxes and metabolite concentrations during rest-to-heavy-work transition are significantly greater. Muscle } \text{VO}_2 \text{ increases 31.9 times, [ADP] increases 4.32 times, pH drops by 0.23, [ATP] is essentially constant, [PCr] decreases to 46.5\% of its initial value, and [Pi] increases 5.17 times (Table 1). An increase in } \tau(\text{OFF}) \text{ from 0 s through 10 s, 30 s, and 100 s to 1,000 s causes an increase in } t_{0.63\text{off}} \text{ for } \text{VO}_2 \text{ from 1.6 s to 11.1 s and decrease in } t_{0.63\text{off}} \text{ for PCr from 225.6 s to 32.6 s (see Table 3). The PCr recovery overshoot appears only at } \tau(\text{OFF}) \text{ of 1,000 s (Fig. 2). } t_{0.63} \text{ for } \text{VO}_2 \text{ is significantly greater for on- than for off-transient for all values of } \tau(\text{OFF}). \text{ } t_{0.63} \text{ for PCr is significantly longer for off- than on-transient at all values of } \tau(\text{OFF}). \text{ The relation between } t_{0.63\text{on}} \text{ for } \text{VO}_2 \text{ and PCr is asymmetrical: the on-transient for PCr is longer than for } \text{VO}_2 \text{ (differently than in moderate exercise). The difference between } t_{0.63\text{off}} \text{ for } \text{VO}_2 \text{ and PCr is already big at } \tau(\text{OFF}) \text{ of 1,000 s and 100 s and becomes very big at } \tau(\text{OFF}) \text{ of 10 s and 0 s (} t_{0.63\text{off}} \text{ for } \text{VO}_2 \text{ is always shorter). It should be noticed that both at moderate and heavy exercise, a faster initial phase of the } \text{VO}_2 \text{ off-kinetics is associated with a slower late phase of this kinetics (see Figs. 1 and 2). As a result, the integral of } \text{VO}_2 \text{ (above the baseline), representing the total oxygen debt, is identical for different values of } \tau(\text{OFF}) \text{ for the same decrease of PCr during work.}

**DISCUSSION**

The main finding of the present theoretical study is that the faster the (initial phase of the) muscle } \text{VO}_2 \text{ off-kinetics, the slower the PCr off-kinetics—and inversely. The inverse relationship between the } \text{VO}_2 \text{ and PCr off-kinetics, although logical, is by no means commonly recognized and accepted. We are not aware of any such conclusion in the literature. On the contrary, it was, for instance, stated in Kemp (9) that “The faster the postexercise inactivation of the parallel activation mechanism, the slower the recovery of PCr and } \text{VO}_2.” \text{ We ourselves, as well as others, overlooked this phenomenon for years, although we had at our disposal an appropriate tool (i.e., our model) to predict it [for instance, we (19) did not present the } \text{VO}_2 \text{ on/off-kinetics].}

Computer simulation carried out in our previous study (19) demonstrated that the higher the value of the } \tau(\text{OFF}), \text{ the shorter the characteristic transition time } t_{0.63\text{off}} \text{ for the off-transient for PCr. This prediction was confirmed re-}

---

Table 2. Characteristic transition times } t_{0.63} \text{ for } \text{VO}_2 \text{ and PCr for on- and off-transient at different values of the characteristic decay time of oxidative phosphorylation activation } \tau(\text{OFF}) \text{ for moderate exercise}

<table>
<thead>
<tr>
<th>Transient</th>
<th>\text{VO}_2</th>
<th>PCr</th>
</tr>
</thead>
<tbody>
<tr>
<td>On-transient</td>
<td>20.0 s</td>
<td>18.5 s</td>
</tr>
<tr>
<td>Off-transient</td>
<td>\tau(\text{OFF}) = 0 s</td>
<td>174.4 s</td>
</tr>
<tr>
<td>\tau(\text{OFF}) = 10 s</td>
<td>112.6 s</td>
<td></td>
</tr>
<tr>
<td>\tau(\text{OFF}) = 30 s</td>
<td>35.3 s</td>
<td></td>
</tr>
<tr>
<td>\tau(\text{OFF}) = 100 s</td>
<td>25.1 s</td>
<td></td>
</tr>
<tr>
<td>\tau(\text{OFF}) = 1,000 s</td>
<td>23.0 s</td>
<td></td>
</tr>
</tbody>
</table>

\( t_{0.63} \) characteristic transition time necessary to undergo 63\% of the final (complete) change to characterize transitions and to compare computer simulations with experimental results; on- and off-transient, rest-to-work transition and work-to-rest transition, respectively.

---

Table 3. Characteristic transition times } t_{0.63} \text{ for } \text{VO}_2 \text{ and PCr for on- and off-transient at different values of the } \tau(\text{OFF}) \text{ for heavy exercise}

<table>
<thead>
<tr>
<th>Transient</th>
<th>\text{VO}_2</th>
<th>PCr</th>
</tr>
</thead>
<tbody>
<tr>
<td>On-transient</td>
<td>15.2 s</td>
<td>23.0 s</td>
</tr>
<tr>
<td>Off-transient</td>
<td>\tau(\text{OFF}) = 0 s</td>
<td>1.6 s</td>
</tr>
<tr>
<td>\tau(\text{OFF}) = 10 s</td>
<td>5.8 s</td>
<td></td>
</tr>
<tr>
<td>\tau(\text{OFF}) = 30 s</td>
<td>8.4 s</td>
<td></td>
</tr>
<tr>
<td>\tau(\text{OFF}) = 100 s</td>
<td>10.0 s</td>
<td></td>
</tr>
<tr>
<td>\tau(\text{OFF}) = 1,000 s</td>
<td>11.1 s</td>
<td></td>
</tr>
</tbody>
</table>

J Appl Physiol • doi:10.1152/japplphysiol.00469.2013 • www.jappl.org
cently, also in the theoretical way, by Kemp (9). However, in both articles, the V\textsubscript{O\textsubscript{2}} on/off-kinetics was not presented explicitly. We also observed that when \(\tau(\text{OFF})\) is high enough, the PCr recovery overshoot appears (13, 19). However, none of these studies concerned the impact of \(\tau(\text{OFF})\) on the V\textsubscript{O\textsubscript{2}} off-kinetics. In the present study, we examined, in parallel, the effect of \(\tau(\text{OFF})\) on the off-kinetics of V\textsubscript{O\textsubscript{2}} and PCr. Computer simulations show that an increase in \(\tau(\text{OFF})\) slows down the initial phase of the V\textsubscript{O\textsubscript{2}} off-kinetics but accelerates the PCr off-kinetics (see Figs. 1 and 2 and Tables 2 and 3). As a consequence, the relationship between these two off-kinetics is inverse: the faster the initial phase of the V\textsubscript{O\textsubscript{2}} off-kinetics, the slower the PCr off-kinetics—and inversely. Therefore, the situation is quite different than in on-transient, where V\textsubscript{O\textsubscript{2}} kinetics and PCr kinetics are approximately mirror images of each other (see, e.g., Figs. 1 and 2).

On the other hand, whereas at the first look, the inverse relationship between the V\textsubscript{O\textsubscript{2}} and PCr off-kinetics may seem unexpected, it is perfectly logical. In the absence of anaerobic glycolysis, oxidative phosphorylation is the only source of ATP for PCr resynthesis during muscle recovery after exercise. When the activity of oxidative phosphorylation, expressed in terms of V\textsubscript{O\textsubscript{2}}, falls down quickly after the termination of exercise, the intensity of PCr resynthesis is slow, which manifests itself as a slow PCr off-kinetics. On the other hand, a slow decay of the activation of oxidative phosphorylation during muscle recovery and thus a slow decrease in V\textsubscript{O\textsubscript{2}} ensure a relatively quick PCr resynthesis and therefore, generate fast PCr off-kinetics. It must be emphasized that the integral of V\textsubscript{O\textsubscript{2}} (above the baseline), representing the total oxygen debt, is identical for different values of \(\tau(\text{OFF})\) for the same decrease of PCr during exercise and thus for the same amount of PCr that is to be resynthesized during recovery. This is because a faster initial phase of the V\textsubscript{O\textsubscript{2}} off-kinetics is associated with a slower late phase of this kinetics (see Figs. 1 and 2). This slower late phase is associated with elevated [ADP] that drives slow PCr resynthesis by oxidative phosphorylation in the absence of parallel activation of this process. This is obvious, because in the absence of anaerobic glycolysis in the recovery period, in all of the cases [different \(\tau(\text{OFF})\) values at a given work intensity], the same amount of oxygen must be used to resynthesize the same amount of PCr. Generally, there is essentially no unique and necessary relationship between V\textsubscript{O\textsubscript{2}} and PCr on- and off-kinetics. It has been shown that the magnitude of the oxygen debt after exercise of a given power output in trained individuals is lower than in untrained subjects (5). This is caused by a smaller PCr decrease during exercise in trained subjects (8), which is, in turn, brought about by elevated mitochondria biogenesis and/or intensification of the parallel activation (each-step activation) of oxidative phosphorylation (13, 17, 20, 36).

Of course, not all values of \(\tau(\text{OFF})\) used in simulations shown in Figs. 1 and 2 lead to predicted muscle V\textsubscript{O\textsubscript{2}} and PCr off-kinetics that are physiologically relevant. The best agreement with experimental results can be observed for the values of \(\tau(\text{OFF})\) between \(\sim 30\) s, resulting in \(t_{0.63}\text{(OFF)}\) for PCr equal to 35.3 s (moderate exercise) or 67.6 s (heavy exercise; see Tables 2 and 3), and 300 s, generating a moderate transient PCr recovery overshoot (13, 19). \(\tau_p\) for PCr off-transient, equal to 42 – 47 s, was observed in young, healthy individuals after heavy exercise (3, 28), and PCr overshoot appears under some conditions (37). In fact, manipulating with \(\tau(\text{OFF})\), we are able to generate any physiologically realistic value of \(t_{0.63}\text{(OFF)}\) for PCr. The slow decay of the activation of oxidative phosphorylation [long \(\tau(\text{OFF})\)], leading to a relatively quick PCr resynthesis during muscle recovery, seems to be beneficial for the organism, because it causes restoration of resting metabolic status in a short time and prepares muscles for a subsequent exercise.

The values of fluxes and metabolite concentrations and their changes during work transitions differ, to some extent, in different experimental systems, muscles, animals, and exercise intensities. However, the simulated values of these variables are well within the range encountered in experimental studies; below are some examples.

In electrically stimulated dog gastrocnemius muscle, V\textsubscript{O\textsubscript{2}} increased 40 and 28 times (to 7.2 and 4.8 mM/min) during on-transitions, ADP increased between 2.5 times and 5.7–7.6 times, PCr decreased to 60% and 32% of its resting value, pH decreased by 0.02 and 0.08 pH units, and \(\tau_p\), for the V\textsubscript{O\textsubscript{2}} on-kinetics, equaled 15.6 s and 35.7 s (see Refs. 35 and 34, respectively).

In severe, submaximal exercise of calf muscles to exhaustion, lasting, on average, 5 min, PCr decreased to approximately 49% and 32% of its initial value, pH decreased by 0.28 and 0.46 pH units in human subjects, without and with PCr recovery overshoot, respectively (37).

In some other studies concerning cycling, PCr content in the quadriceps dropped to approximately 53%, 43%, and 20% of the initial value, whereas muscle pH decreased by approximately 0.13, 0.14, and 0.30 pH units, with exercise intensity corresponding to approximately 65%, 75%, and 90% of V\textsubscript{O\textsubscript{2}max}, respectively (6, 29).

Generally, by applying different combinations of the extent of direct activation of ATP use, oxidative phosphorylation, and glycolysis in computer simulations, we are able to reproduce essentially all combinations of the increase in V\textsubscript{O\textsubscript{2}}, decrease in PCr, and decrease in pH encountered in experimental studies during on-transitions in skeletal muscle (not shown).

The pulmonary characteristic transition \(\tau_\text{p}\) of the fundamental component of the V\textsubscript{O\textsubscript{2}} on-kinetics in humans during heavy exercise, amounting to 15–40 s [on average, 25.5 s; see Table 12.2 in Poole and Jones (24)], is slightly longer than the muscle \(t_{0.63}\text{(on)}\), equal to 20.0 s for moderate exercise and 15.2 s for heavy exercise in our simulations. However, first, they are within the reported range. Second, whereas \(\tau_\text{p}\) is similar for muscle and pulmonary V\textsubscript{O\textsubscript{2}} on-transient during moderate exercise (4), it is likely that they diverge at heavy exercise because of delays in oxygen transport by blood. Third, the simulated \(t_{0.63}\text{(on)}\) for heavy exercise agrees well with the muscle \(\tau_\text{p}\) for on-transient, measured in electrically stimulated dog gastrocnemius muscle (15.6 s) (35). Fourth, a rather high intensity of direct parallel activation of oxidative phosphorylation, characterized by the power coefficient \(p\) equal to 0.4, was assumed in computer simulations in the present study. When a lower parallel activation intensity, for instance, \(p = 0.3\), is applied, the simulated \(t_{0.63}\text{(on)}\) is much longer [compare, for instance, Korzeniewski (13) and Korzeniewski and Zoladz (17, 20)]. This fact and the difference of the behavior of the system between moderate and heavy exercise, simulated in the present study, suggest that not only \(\tau(\text{OFF})\) but also the
work intensity and parallel activation of oxidative phosphorylation intensity significantly influence the mutual relations between VO₂ and PCr on- and off-kinetics. The impact of the τ(ON) seems to be of lower importance, because its value is significantly lower than t₀.₆₃₉₉ for VO₂ [compare also Wüst et al. (34)].

In our simulations, t₀.₆₃₉₉ for muscle VO₂ is shorter for heavy than for moderate exercise, whereas this is not what is observed for pulmonary VO₂ in experimental studies: the VO₂ on-kinetics for moderate exercise is equally fast (23) or faster (24) than for heavy exercise. However, first, as discussed above, the pulmonary and muscle VO₂ on-kinetics can diverge at heavy exercise. Second, we assumed for simplicity the same value (0.4) of the power coefficient p (that characterizes the intensity of parallel activation) for moderate and heavy exercise. This gives a high, direct activation of oxidative phosphorylation during heavy exercise equal to \(80^{0.4} = 5.77\). If a lower value of p is assumed for heavy exercise, then the predicted t₀.₆₃₉₉ for heavy exercise will be equal to or longer than for moderate exercise (not shown).

In our simulations, the muscle t₀.₆₃₉₉ for VO₂ is essentially the same as t₀.₆₃₉₉ (off-on symmetry) for moderate exercise at τ(OFF) = 1,000 s (see Table 2). This prediction agrees well with the only experimental study concerning muscle VO₂ for low work intensity (2). However, at lower values of τ(OFF), t₀.₆₃₉₉ for VO₂ was longer than t₀.₆₃₉₉. A direct comparison of the simulated muscle VO₂ on-off relationship with the experimental pulmonary VO₂ on-off relationship does not make much sense, because it is likely that ongoing restitution after exercise, involving elevated heart rate and respiratory muscle activity, can contribute, along with decreasing blood flow, to slowing down the pulmonary VO₂ off-kinetics in relation to locomotory muscle VO₂ off-kinetics. Anyway, t₀.₆₃₉₉ for muscle VO₂ depends strongly on the τ(OFF), as it can be seen in Figs. 1 and 2 and Tables 2 and 3.

Computer simulations predict that t₀.₆₃₉₉ is shorter for VO₂ (15.2 s) than for PCr (23.0 s) at heavy exercise. On the other hand, similar values of on-transient τₚ for pulmonary VO₂ and PCr at both moderate and heavy exercise were measured by Rossiter and coworkers (28), as in our simulations for moderate exercise. However, again, it is likely that limitations of blood circulation, especially in heavy exercise, cause a mismatch between pulmonary and muscle VO₂ on-kinetics. This supposition is confirmed by the recent study by Wüst and coworkers (34) on electrically stimulated dog gastrocnemius muscle, showing that muscle τₚ of the VO₂ on-kinetics was much shorter than the τₚ of the PCr on-kinetics (35.7 s or 47.1 s vs. 62.5 s).

In computer simulations concerning both moderate and especially heavy exercise, t₀.₆₃₉₉ is much shorter for muscle VO₂ than for PCr. On the other hand, the values of off-transient τₚ seem to be similar for pulmonary VO₂ and PCr, both in moderate and heavy exercise in humans (28). However, as discussed above, it is likely that there is a significant mismatch between the pulmonary and muscle VO₂ off-kinetics, especially in heavy exercise.

In many aspects, the model used in the present study is certainly only semiquantitative. It is intended to reproduce approximately (changes in) many different variables, measured in a broad range of experiments, concerning different muscles/animals and various experimental conditions and not to reproduce strictly quantitatively (changes in) one or few variables in one particular experiment.

For simplicity, we do not take into account AMP deamination, and therefore, ATP remains essentially constant in our simulations. This process takes place in severe exercise, at which, a significant decrease in PCr and pH takes place [see, e.g., Taylor et al. (30)]. The effect of AMP deamination on the skeletal muscle cell bioenergetic system during severe exercise was analyzed in a previous in silico study (10). We do not take into account the slow component of the VO₂ on-kinetics, expressing a progressive increase of energy cost of generation of muscle force or power output at high exercise intensity [for an overview of this point, see, e.g., Jones et al. (7)]. We modeled the slow component in our previous theoretical article (18).

It must be stressed that the inverse dependence between the VO₂ and PCr off-kinetics, predicted in the present study, requires both parallel activation (each-step activation) of oxidative phosphorylation during exercise and a slow (not instant) decay of this activation during muscle recovery. As it was discussed broadly elsewhere (11, 14), only computer models involving direct parallel activation of all oxidative phosphorylation complexes can explain satisfactorily a broad range of the muscle bioenergetic system properties during work transitions, whereas other models [see, e.g., Wu et al. (33)] fail to do so. Some models that did not involve the parallel activation mechanism and therefore, assumed exclusively the feedback mechanism (mediated by PCr, ADP, and/or P_i) were used to study the PCr on- and off-kinetics (21, 22, 31). The authors did not model (or present) explicitly the VO₂ on- and off-kinetics, although the ATP synthesis rate was modeled in Kushmerick (21). Generally, these models could predict only one unique VO₂ (or ATP synthesis) off-kinetics for a given work intensity, because they, on principle, did not involve (different values of) τ(OFF). Additionally, we believe that in the experimental system of electrically stimulated gastrocnemius in anesthetized rats, modeled by Meyer (22), there could be, in fact, no parallel activation [see Korzeniewski (13) for discussion]. The author predicted that the transition time for PCr for on- and off-transient is long (\(~1.5\) min) and that the off-transient is equally as long as, or slightly longer than, the on-transient, depending on the stimulation frequency. A similar theoretical result was obtained by Kushmerick (21). If it is assumed that there is no parallel activation in our model, the theoretical predictions of the model are also very similar [compare Fig. 1 in Korzeniewski (13) vs. Fig. 4 in Meyer (22) and Fig. 3 in Kushmerick (21)]. Vicini and Kushmerick (31) modeled PCr on-transient during anoxia, which is irrelevant for the present considerations.

In conclusion, in the absence of anaerobic glycolysis during the muscle recovery process, the τ(OFF) during muscle recovery determines, to a large extent, the muscle VO₂ and PCr off-kinetics—its high value slows down the initial phase of the former and accelerates the latter. As a result, the relationship between the initial phase of the muscle VO₂ off-kinetics [lasting approximately 3–60 s for the range of τ(OFF) used in the present theoretical study] and the PCr off-kinetics is inverse: the slower the initial phase of the muscle VO₂ off-kinetics, the faster the PCr off-kinetics—and inversely. A faster initial phase of the VO₂ off-kinetics is associated with a slower late phase of the VO₂ off-kinetics (after approximately 3–60 s). As
a result, the integral of \( \dot{V}O_2 \) above baseline during recovery, representing the oxygen debt, is identical for all values of \( \tau \) (OFF) for the same PCr decrease during exercise. Generally, \( \tau \) (OFF) has a pronounced impact on the mutual relations (for instance, symmetry or asymmetry) between the muscle \( \dot{V}O_2 \) and PCr on- and off-kinetics.

**APPENDIX**

This is a concise, complete kinetic description of the dynamic model of oxidative phosphorylation plus anaerobic glycolysis in intact skeletal muscle. Subscripts: e, external (cytosolic); i, internal (mitochondrial); t, total; f, free; m, magnesium complex; j, monovalent.

**Kinetic Equations**

All reaction rates are expressed in \( \mu M/min \).

Substrate dehydrogenation

\[
v_{DH} = k_{DH} \frac{1}{1 + \frac{K_{mN}}{[NAD]/[NADH]}}
\]

\( k_{DH} = 28,074 \ \mu M/min, \ K_{mN} = 100, \ p_D = 0.8 \)

**Complex I**

\[ v_{C1} = k_{C1} \cdot \Delta G_{C1} \]

\( k_{C1} = 238.95 \ \mu M \cdot mV^{-1} \cdot min^{-1} \)

**Complex III**

\[ v_{C3} = k_{C3} \cdot \Delta G_{C3} \]

\( k_{C3} = 136.41 \ \mu M \cdot mV^{-1} \cdot min^{-1} \)

**Complex IV**

\[ v_{C4} = k_{C4} \cdot a^{2+} \cdot c^{3+} \frac{1}{1 + \frac{K_{mO}}{[O_2]}} \]

\( k_{C4} = 3.600 \ \mu M/min, \ K_{mO} = 120 \ \mu M \) (apparent \( K_{mO} \) = about 0.8 \( \mu M \), depending on conditions)

ATP synthase

\[ v_{SN} = k_{SN} \cdot \frac{\gamma - 1}{\gamma + 1} \]

\( k_{SN} = 34,316 \ \mu M/min, \ \gamma = 10^{A_{GCD/Z}} \)

ATP/ADP carrier

\[ v_{EX} = k_{EX} \cdot \left( \frac{ADP_{te}}{ADP_{te} + ATP_{te} \cdot 10^{-9} [Z]} - \frac{ADP_{fi}}{ADP_{fi} + ATP_{fi} \cdot 10^{-9} [Z]} \cdot \frac{1}{1 + K_{mADP}/ADP_{te}} \right) \]

\( \dot{H}^+ = \left( \frac{2 \cdot (2 + 2 \cdot u) \cdot v_{C4} + (4 - 2 \cdot u) \cdot v_{C3} + 4 \cdot v_{C1} - n_A \cdot v_{SN} - u \cdot v_{EX} - (1 - u) \cdot v_{PI} - v_{LK} - \dot{P}_{Bu}}{s \cdot v_{CK} - v_{EFF} + v_{GLY} + 0.2 \cdot v_{DEHY}} \right) \cdot \eta_{Bu} \)

**Set of Differential Equations**

\[
NAD^+ = (v_{DH} - v_{C1}) \cdot R_{cm} \cdot B_N
\]

\[
UQH_2 = (v_{C1} - v_{C2}) \cdot R_{cm}
\]

\[
c^{2+} = (v_{C2} - 2 \cdot v_{C3}) \cdot 2 \cdot R_{cm}
\]

\( \dot{O}_2 = 0 \) (constant oxygen concentration = 240 \( \mu M \)) or

\( \dot{O}_2 = - v_{C4} \)

\( \dot{H}_e^+ = -(2 \cdot (2 + 2 \cdot u) \cdot v_{C4} + (4 - 2 \cdot u) \cdot v_{C3} + 4 \cdot v_{C1} - n_A \cdot v_{SN} - u \cdot v_{EX} - (1 - u) \cdot v_{PI} - v_{LK} - \dot{P}_{Bu} \)

\( \dot{P}_{i} = (v_{PI} - v_{SN}) \cdot R_{cm} \)

\( \dot{ATP}_{te} = (v_{SN} - v_{EX}) \cdot R_{cm} \)

\( \dot{ATP}_{fe} = v_{EX} - v_{UT} + v_{AK} + v_{CK} + 1.5 \cdot v_{GLYC} \)

\( \dot{ADP}_{te} = v_{UT} - v_{EX} - 2 \cdot v_{AK} - v_{CK} - 1.5 \cdot v_{GLYC} \)

\( \dot{PCr} = - v_{CK} \)

Calculations

\( c^{3+} = c_t - c^{2+} \)

\( v_{PI} = k_{PI} \cdot (P_{je} \cdot H_e - P_{ji} \cdot H_i) \)

\( k_{PI} = 69.421 \ \mu M/min \)

Phosphate carrier

\( v_{UT} = k_{UT} \cdot \frac{1}{1 + \frac{k_{mA}}{ATP_{te}}} \)

\( k_{UT} = 686.50 \ \mu M/min \) (resting state), \( k_{mA} = 150 \ \mu M \)

Proton leak

\( v_{LK} = k_{LK1} \cdot (e^{4partial} - 1) \)

\( k_{LK1} = 2.500 \ \mu M/min, k_{LK2} = 0.038 \) mV^{-1}

Adenylate kinase

\( v_{AK} = k_{fAK} \cdot ADP_{te} \cdot ADP_{me} - k_{bAK} \cdot ATP_{me} \cdot AMP_e \)

\( k_{fAK} = 862.10 \ \mu M/min, k_{bAK} = 22.747 \ \mu M/min \)

Glycolysis

\( v_{GLYC} = k_{GLYC} \cdot ADP_{te} \cdot (H_c^{rest}/H^+) \)

\( k_{GLYC} = 17.31 \) min^{-1}, \( H^+_{rest} = 0.1 \) \mu M

R_{cm} = 15 \ (cell volume/mitochondria volume ratio)

B_N = 5 (buffering capacity coefficient for NAD)

\( s = 0.63 - (pH 6.0) - 0.43 \) \ (net stoichiometry of proton consumption/production by CK when coupled with ATP consumption/production, respectively (Lohmann reaction)).

\( c_t = 270 \ \mu M (=c^{2+} + c^{3+}, \ total \ concentration \ of \ cytochrome \ c) \)

\( UQ = U_t - UQH_2 \)

\( U_t = 1,350 \ \mu M (=UQH_2 + UQ, \ total \ concentration \ of \ ubiquinone) \)

\( NAD^+ = N_t - NAD^+ \)

\( N_t = 2,970 \ \mu M (=NADH + NAD^+, \ total \ concentration \ of \ NAD) \)

\( AMP_e = A_{SUM} - ATP_{te} - ADP_{te} \)

\( A_{SUM} = 6,700.2 \ \mu M (=ATP_{te} + ADP_{te} + AMP_e, \ total \ external \ adenine \ nucleotide \ concentration) \)

**J Appl Physiol • doi:10.1152/japplphysiol.00469.2013 • www.jappl.org**
ADP_n = A_SUM - ATP_n
A_SUM = 16,260 μM (=ATP_n + ADP_n, total internal adenine nucleotide concentration)
Cr = C_SUM - PCr
C_SUM = 35,000 μM (=Cr + PCr, total creatine concentration)
PSUM = 55,659 μM [=PCr + 3 ATP_n + 2 ADP_n + AMP_n + Pi_n + (3ATP_n+2ADP_n+Pi_n)/R_cm (total phosphate pool)]
Mg_fe = 4,000 μM (free external magnesium concentration)
ATP_n = ATP_n(1 + Mg_fe/k_DTE)
K_DTE = 24 μM (magnesium dissociation constant for external ATP)
ATP_mec = ATP_n - ADP_n
ADP_mec = ADP_n(1 + Mg_fe/k_DDE)
K_DDE = 347 μM (magnesium dissociation constant for external ADP)
ADP_n = ADP_mec - ADP_n
Mg_fih = 380 μM (free internal magnesium concentration)
ATP_n = ATP_n(1 + Mg_fih/k_DTTI)
K_DTTI = 17 μM (magnesium dissociation constant for internal ATP)
ATP_n = ATP_n - ADP_n
ADP_n = ADP_n(1 + Mg_fih/k_DDPi)
K_DDPi = 282 μM (magnesium dissociation constant for internal ADP)
ADP_mec = ADP_n - ADP_n
T = 298 K
R = 0.0083 kJ mol⁻¹ K⁻¹
F = 0.0965 kJ mol⁻¹ mV⁻¹
S = 2.303 ± R × T
Z = 2.303 ± R × T/F
u = 0.861 (=ΔV/Δp)

ΔpH = -log(H+/1.000,000) (H⁺ expressed in μM)

Δψ_c = (10⁻ψ_c - 10⁻ψ_c-△ψ_c)/dpH (“natural” buffering capacity for H⁺ in matrix)

Δψ_c = 0.001

Δψ_c = 0.022 M H⁺/pH unit (buffering capacity for H⁺ in matrix)

Csum = (10⁻ψ_c - 10⁻ψ_c-△ψ_c)/dpH (natural buffering capacity for H⁺ in cytosol)

Δψ_c = 0.001

ΔG_ATP = nα * Δψ_c - ΔG_P (thermodynamic span of ATP synthase)

ΔG_P = ΔG_P/O + Z * log [1,000,000 + ATP_n/ADP_n - Pi_n] (concentrations expressed in μM)

nα = 2.5 (phenomenological H⁺/ATP stoichiometry of ATP synthase)

ΔG_P/O = 31.9 kJ/mol

ΔG_NAD = E_MnN + Z/2 * log (NAD⁺/NADH) (NAD redox potential)

E_MnN = -320 mV

E_MnO = E_MnO + Z/2 * log (UQ/UQH₂) (ubiquinone redox potential)

E_MnO = 85 mV

E_mco = E_mco + Z * log (c⁺/c⁻²⁺) (cytochrome c redox potential)

E_mco = 250 mV

E_mco = E_mco + Δψ_c (cytochrome a₃ redox potential)

E_mco = 10 kTm - E_MnO/α_bar (α_bar = α₂/α₃ ratio)

α₂ = α/(1 + a₂/α)

a₂ = 135 μM

E_mco = 540 mV

ΔG_C1 = E_mti - E_mN - Δψ_c * 4/2 (thermodynamic span of complex I)

ΔG_C2 = E_mti - E_mti - Δψ_c * (4 - 2) μV/2 (thermodynamic span of complex III)

GRANTS

Support for J. A. Zoladz was provided by the Ministry of Science and Higher Education (Poland), Grant No. N N404 196637.

DISCLOSURES

The authors declare no conflicts of interest.

ENDNOTE

At the request of the authors, readers are herein alerted to the fact that additional materials related to this manuscript may be found at the institutional website of one of the authors, which at the time of publication, they indicate is: [http://www.molphys.edu.pl/~benio]. These materials are not a part of this manuscript and have not undergone peer review by the American Physiological Society (APS). APS and the journal editors take no responsibility for these materials, for the website address, or for any links to or from it.

AUTHOR CONTRIBUTIONS

Author contributions: B.K. and J.A.Z. conception and design of research; B.K. performed experiments; B.K. analyzed data; B.K. and J.A.Z. interpreted results of experiments; B.K. prepared figures; B.K. and J.A.Z. drafted manuscript; B.K. and J.A.Z. edited and revised manuscript; B.K. and J.A.Z. approved final version of manuscript.

REFERENCES


