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Authors
Korzeniewski, Bernard
Rossiter, Harry B
Zoladz, Jerzy A

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Mechanisms underlying extremely fast muscle $\dot{V}O_2$ on-kinetics in humans

Bernard Korzeniewski¹, Harry B. Rossiter²,³ & Jerzy A. Zoladz⁴

¹ BioSimulation Center, Kraków, Poland
² Rehabilitation Clinical Trials Center, Division of Pulmonary Critical Care Physiology and Medicine, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, California
³ Faculty of Biological Sciences, University of Leeds, Leeds, United Kingdom
⁴ Department of Muscle Physiology, Chair of Physiology and Biochemistry, Faculty of Rehabilitation, University School of Physical Education, Kraków, Poland

Abstract

The time constant of the primary phase of pulmonary $\dot{V}O_2$ on-kinetics ($\tau_p$), which reflects muscle $\dot{V}O_2$ kinetics during moderate-intensity exercise, is about 30 s in young healthy untrained individuals, while it can be as low as 8 s in endurance-trained athletes. We aimed to determine the intramuscular factors that enable very low values of $t_{0.63}$ to be achieved (analogous to $\tau_p$, $t_{0.63}$ is the time to reach 63% of the $\dot{V}O_2$ amplitude). A computer model of oxidative phosphorylation (OXPHOS) in skeletal muscle was used. Muscle $t_{0.63}$ was near-linearly proportional to the difference in phosphocreatine (PCr) concentration between rest and work ($D_{PCr}$). Of the two main factors that determine $t_{0.63}$, a huge increase in either OXPHOS activity (six- to eightfold) or each-step activation (ESA) of OXPHOS intensity ($>3$-fold) was needed to reduce muscle $t_{0.63}$ from the reference value of 29 s (selected to represent young untrained subjects) to below 10 s (observed in athletes) when altered separately. On the other hand, the effect of a simultaneous increase of both OXPHOS activity and ESA intensity required only a twofold elevation of each to decrease $t_{0.63}$ below 10 s. Of note, the dependence of $t_{0.63}$ on OXPHOS activity and ESA intensity is hyperbolic, meaning that in trained individuals a large increase in OXPHOS activity and ESA intensity are required to elicit a small reduction in $\tau_p$. In summary, we postulate that the synergistic action of elevated OXPHOS activity and ESA intensity is responsible for extremely low $\tau_p$ ($t_{0.63}$) observed in highly endurance-trained athletes.

Introduction

The exponential time constant $\tau$ of phase 2 of oxygen consumption ($\dot{V}O_2$) on-kinetics in skeletal muscle (i.e., the time to reach 63% of the $\dot{V}O_2$ amplitude, termed $\tau_p$, or $t_{0.63}$) is the fundamental parameter characterizing the muscle bioenergetic system (Grassi et al. 1996; Whipp and Rossiter 2005; Zoladz et al. 2014). During moderate-intensity (i.e., below the lactate threshold) whole-body exercise such as cycling, muscle $\dot{V}O_2$ on-kinetics is well reflected by the time constant of phase 2 pulmonary $\dot{V}O_2$ on-kinetics ($\tau_p$) (Grassi et al. 1996). In young healthy untrained individuals during moderate-intensity exercise $\tau_p$ approximately ranges 25–40 s (Whipp and Rossiter 2005; Grassi et al. 2011; Zoladz et al. 2013), while in well-trained endurance athletes it can be as low as 8 s.
exercise, with the result that pulmonary \( \dot{V}_{\text{O}_2} \) may be compromised during the first minutes of either oxygen delivery and muscle oxygen extraction may be compromised for the result that pulmonary \( \tau_p \), can exceed 60 s (Grassi et al. 2011). Pathologically slow kinetics is predictive of poor prognosis (Schalcher et al. 2003). Pulmonary phase 2 \( \dot{V}_{\text{O}_2} \) kinetics is also slow in mitochondrial diseases and McArdle’s disease (Grassi et al. 2009), where oxidative phosphorylation (OXPHOS) activity is compromised.

Several animal-based and human experiments have supported the notion that muscle \( \dot{V}_{\text{O}_2} \) on-kinetics during moderate-intensity exercise in healthy young subjects is not limited by convective or diffusive oxygen delivery (Grassi et al. 1998; Bangsbo et al. 2000; Grassi 2005; Nyberg et al. 2014; Richardson et al. 2015). Most of the available experimental evidence suggests that muscle \( \dot{V}_{\text{O}_2} \) on-kinetics is mainly controlled or limited by intramuscular factors related to metabolic activation (Grassi et al. 2011; Rossiter 2011; Wüst et al. 2011; Poole and Jones 2012). However, the factors determining the value of muscle \( \tau_p \) are not fully understood.

Previous theoretical studies demonstrated that two main factors determine muscle \( \dot{V}_{\text{O}_2} \) on-kinetics: OXPHOS activity and each-step activation (ESA) intensity (Korzeniewski and Zoladz 2004). ESA was proposed as the main mechanism responsible for the regulation of OXPHOS during work transitions in skeletal muscle and heart (Korzeniewski 1998, 2007, 2017; Zoladz et al. 2013, 2014; Korzeniewski and Rossiter 2015). This mechanism was first named “parallel activation” (Korzeniewski 1998), but afterwards renamed “each-step activation” in order to avoid confusion with other mechanisms involving simultaneous activation of ATP usage and ATP supply (Korzeniewski 2007; Korzeniewski and Rossiter 2015). A parallel activation of ATP usage and ATP supply block in general was first proposed by Hochachka (1994). On the basis of theoretical studies, ESA is characterized by direct activation of all OXPHOS complexes (complex I, complex III, complex IV, ATP synthase, ATP/ADP carrier, and P\(_i\) carrier), the NADH supply block, and glycolysis, in parallel with the activation of ATP usage during rest-to-work or low-to-high work transitions.

These computational simulations showed that muscle \( \dot{V}_{\text{O}_2} \) on-kinetics, using values typical of healthy young subjects, could be reasonably predicted on the basis of the known biochemical properties and the difference between resting and exercising PCR concentration (\( \Delta \text{PCR} \)) (Korzeniewski and Zoladz 2004). However, these simulations interrogated only a relatively narrow range of OXPHOS activity and ESA intensity, and only the independent effects of either OXPHOS activity or ESA intensity were studied; not the synergistic effect of both these factors acting simultaneously.

Muscle mitochondrial volume (density) is about twofold greater in highly trained individuals (e.g., \( \dot{V}_{\text{O}_2\text{max}} \) 70–80 mL min\(^{-1}\) kg\(^{-1}\)) compared to average young healthy untrained individuals (\( \dot{V}_{\text{O}_2\text{max}} \) 40–50 mL min\(^{-1}\) kg\(^{-1}\)) (Hoppeler et al. 1973). A more recent study (Larsen et al. 2012) found a ∼3-fold range of muscle mitochondrial volume (density) by transmission electron microscopy, OXPHOS complex concentration by protein quantification, and OXPHOS activity by respirometry, in a group of individuals with \( \dot{V}_{\text{O}_2\text{max}} \) ranging 30–72 mL min\(^{-1}\) kg\(^{-1}\).

We therefore studied the effect of large changes in OXPHOS activity and ESA intensity on \( \Delta \text{PCR} \) and muscle \( t_{0.63} \) to determine the skeletal muscle bioenergetic system characteristics required to elicit very low \( \tau_p \) values observed in athletes. Both the separate effect of each factor and the synergistic effect of each acting simultaneously were analyzed. It was hypothesized that a huge increase in either OXPHOS activity or ESA intensity separately is necessary to decrease muscle \( t_{0.63} \) below 10 s, while only a moderate increase is sufficient when the magnitudes of both factors are elevated simultaneously. It was also hypothesized that the dependence of muscle \( t_{0.63} \) on OXPHOS activity (kOX) and ESA intensity (A\( \text{OX} \)) is hyperbolic rather than linear, and therefore \( t_{0.63} \) would be much more sensitive to changes in kOX and A\( \text{OX} \) at low kOX and A\( \text{OX} \) values, than at high kOX and A\( \text{OX} \) values.

### Theoretical Methods

#### Computer model

The well-tested computer model of OXPHOS and the entire bioenergetic system in intact skeletal muscle was used in the simulations carried out in this study (Korzeniewski and Zoladz 2001; Korzeniewski and Liguzinski 2004). This model comprises explicitly NADH supply block (TCA cycle, fatty-acid \( \beta \)-oxidation, MAS, etc.), particular OXPHOS complexes (complex I, complex III, complex IV, ATP synthase, ATP/ADP carrier, and P\(_i\) carrier), the NADH supply block, and glycolysis, in parallel with the activation of ATP usage during rest-to-work or low-to-high work transitions.

These computational simulations showed that muscle \( \dot{V}_{\text{O}_2} \) on-kinetics, using values typical of healthy young subjects, could be reasonably predicted on the basis of the known biochemical properties and the difference between resting and exercising PCR concentration (\( \Delta \text{PCR} \)) (Korzeniewski and Zoladz 2004). However, these simulations interrogated only a relatively narrow range of OXPHOS activity and ESA intensity, and only the independent effects of either OXPHOS activity or ESA intensity were studied; not the synergistic effect of both these factors acting simultaneously.
Computer simulations

The two main parameters used in this study can be defined as follows. Relative OXPHOS activity (kOX) is the unitless basal value of the rate constants of all OXPHOS complexes expressed relative to 1 in the reference (standard) state. kOX is the relative OXPHOS activity in the absence of ESA. ESA intensity (Aox) is the unitless fold increase in the values of the rate constants of all OXPHOS complexes during rest-to-work transition equal to 4.5 in the reference (standard) state. Therefore, kOX is the relative OXPHOS activity at rest and kOX x Aox is the relative OXPHOS activity during work. Neither kOX, constituting the relative rate constant of OXPHOS at rest, nor kOX x Aox, representing the relative rate constant of OXPHOS during work, is equivalent to the absolute flux of muscle oxygen consumption (VO2, or its equivalent ATP synthesis flux) expressed in mmol/L min−1. VO2 depends not only on absolute OXPHOS activity (rate constant) but also on metabolite concentrations, for example, of ADP and P. kOX is, of course, related to mitochondrial density as well as to the concentration and activity of OXPHOS complex enzymes.

Moderate exercise was analyzed in this study, in which the activity of ATP usage was 30 times higher than at rest. This rate is anticipated to be approximately equivalent to about 30–35% of the muscle VO2max measured during cycle ergometry. This corresponds to about 40% of the pulmonary VO2max during cycling. The exact work intensity applied is of minor importance, as τp depends little on work intensity in constant-power exercise (Poole and Jones 2005). The reference state used for the simulations corresponded to that of young healthy untrained people (with pulmonary VO2max = 40–50 mL min−1 kg−1 and τp = 29.2 s) (e.g., Hoppeler et al. 1973; Rossiter 2011). The standard OXPHOS activity appearing in the model (the reference state) is indicated as “kOX * 1”, that is, onefold of the standard OXPHOS activity increase. In order to obtain muscle t0.63 equal to about 30 s during moderate work for the reference state, an ESA intensity (Aox) equal to 4.5 was required. In this reference state the muscle VO2 = 3.62 mmol/L min−1, resting PCr = 27.74 mmol/L, ΔPCr = 10.80 mmol/L, and muscle t0.63 = 29.2 s.

In subsequent simulations either OXPHOS activity or ESA intensity was changed separately, or both were changed simultaneously. An n-fold increase in the resting (without ESA) OXPHOS activity (kOXn) was equivalent to an n-fold increase in resting rate constants of all OXPHOS complexes and NADH supply (kC1, kC2, kC4, kSN, kEX, kPD, and kDH). The resting OXPHOS activity was either increased (n > 1) or decreased (n < 1) in relation to the reference state. Proton leak activity was not changed, as it was assumed that the increase in the proton leak activity related to the twofold greater mitochondrial volume between the reference state (young healthy untrained) and the endurance-trained state (with pulmonary VO2max above 70 mL min−1 kg−1 and τp < 10 s) is compensated by a training-induced twofold decrease in proton leak intensity per mitochondrial volume. This assumption is partly based on experimental data illustrating the magnitude of the endurance training-induced mitochondrial biogenesis and oxidative capacity, was accompanied by a decrease in mitochondrial uncoupling, including uncoupling protein-mediated proton leak studied in rat skeletal muscle mitochondria (Zoladz et al. 2016). A particular value of ESA intensity (Aox) meant that the rate constants of all OXPHOS complexes and NADH supply (kC1, kC2, kC4, kSN, kEX, kPD, and kDH) were elevated Aox times during rest-to-work transitions. Aox was either increased (Aox > 4.5) or decreased (Aox < 4.5) in relation to the reference state.

Results of Simulations

This is a purely theoretical work and does not involve experiments on humans or animals.

Figure 1 demonstrates that muscle t0.63 had a near-linear dependence on the difference between PCr at rest and during exercise (ΔPCr). This relationship was independent of whether ΔPCr was changed through a change in the resting OXPHOS activity (kOX), that is, without ESA, or through a change in ESA intensity (Aox). However, when the resting OXPHOS activity and ESA intensity were modified separately, a huge increase in these parameter values was necessary in order to diminish muscle t0.63 significantly from the reference state value of 29.2 s. Specifically, an approximate sixfold increase in resting OXPHOS activity was required in order to decrease muscle t0.63 to 10 s and an eightfold increase was necessary to reach muscle t0.63 = 8 s. Alternatively, it was necessary to increase ESA intensity 3.1-fold (from Aox = 4.5 to Aox = 14) to diminish muscle t0.63 to 8 s.

Both resting (without ESA) OXPHOS activity and ESA intensity affect the overall OXPHOS activity during muscle work. However, resting OXPHOS activity affects the resting PCr concentration, while ESA intensity does not: Aox acts only during muscle work. This is why the former must be elevated eightfold and the latter only 3.1-fold in order to evoke the same decrease in muscle t0.63 (to 8 s). An increase in resting OXPHOS activity demands an elevation in resting PCr concentration. This in turn requires an increased ΔPCr and thus slows down muscle t0.63. Therefore, a relatively high increase in the resting OXPHOS activity kOX is necessary in order to diminish muscle t0.63 below 10 s. This effect is absent in
case of increasing ESA intensity, and therefore adjusting ESA intensity was more effective in decreasing $t_{0.63}$ than an adjustment in resting (without ESA) OXPHOS activity.

The requirement for a huge increase in resting (without ESA) OXPHOS activity and, to a lesser extent, ESA intensity could be avoided by using the synergistic effect of a simultaneous increase in the magnitude of both factors together. This is demonstrated in Figure 2. A parallel twofold increase in resting (without ESA) OXPHOS activity ($k_{OX}$) and ESA intensity ($A_{OX}$) (increasing $A_{OX}$ from 4.5 to 9) leads to a decrease of muscle $t_{0.63}$ from 29 s in the reference state to 9 s in the “activated” state. Therefore, the synergistic effect of increasing both resting OXPHOS activity and ESA intensity was very effective in decreasing the $t_{0.63}$ of muscle $\dot{V}O_2$.

Simulated dependence of muscle $t_{0.63}$ on relative reference OXPHOS activity ($k_{OX}$), relative ESA intensity ($A_{OX}$), and the product of $k_{OX}$ and $A_{OX}$ is essentially hyperbolic. This is demonstrated in Figure 3. These dependencies are extracted from Figures 1 and 2.

The fact that $\Delta PCr$ between rest and work is strictly related to muscle $t_{0.63}$ is illustrated in Figure 4. One can see that when both resting OXPHOS activity and ESA intensity are elevated in relation to the reference state, $\Delta PCr$ is much smaller and $\dot{V}O_2$ kinetics is speeded compared with the reference state. On the other hand, when the magnitudes of both factors are decreased, $\Delta PCr$ is significantly greater and $\dot{V}O_2$ kinetics is significantly slowed in relation to the reference state.

**Discussion**

This theoretical intramuscular study demonstrates that: (1) both an increase in resting (without ESA) OXPHOS activity and ESA intensity can significantly accelerate the muscle
VO2 on-kinetics (reduce \( t_{0.63} \)); (2) muscle \( t_{0.63} \) is near-linearly proportional to the difference in the PCR concentration between work and rest (\( \Delta \text{PCR} \)); (3) the muscle \( t_{0.63} \); \( \Delta \text{PCR} \) dependence is identical regardless of whether \( \Delta \text{PCR} \) is changed through an increase in OXPHOS activity or through an increase in ESA intensity; (4) when the magnitudes of OXPHOS activity or in ESA intensity are increased separately, a huge increase in resting OXPHOS activity (six- to eightfold) or ESA intensity (>3-fold) is necessary to decrease the muscle \( t_{0.63} \) from 29 s in the reference state (young healthy individuals) to 8 s (as observed in the extreme endurance-trained state); (5) the required increasing resting OXPHOS activity of >6-fold to decrease the muscle \( t_{0.63} \) to <10 s exceeds greatly the observed increases with endurance training in muscle mitochondrial volume (density) of two- to threefold; and (6) a synergistic effect of a simultaneous increase in resting OXPHOS activity and ESA intensity together requires only twofold increases in the magnitudes of these factors to decrease \( t_{0.63} \) from 29 to 8 s. Therefore, we postulate that the very short \( t_{p} \) (\( t_{0.63} \)) of the muscle and pulmonary VO2 on-kinetics encountered in very highly endurance-trained athletes are due to a high mitochondrial volume (density) related to high (resting) OXPHOS activity as well as a high ESA intensity. Each can result from both genetic factors (interindividual differences) and endurance training.

It is demonstrated that the dependence of the muscle \( t_{0.63} \) on OXPHOS activity and ESA intensity is essentially hyperbolic, as shown in Figure 3. A hyperbolic relationship between \( t_{0.63} \) and whole-body VO2max in different mammal species was previously described (Poole and Jones 2012). Here, we show that, in the context of muscle VO2, independent of oxygen delivery, this hyperbolic relationship is anticipated with training-induced increases in OXPHOS activity and ESA intensity alone, even in the absence of increased mitochondrial volume (density). This implies that a training-induced increase in mitochondrial biogenesis and/or ESA intensity above the reference state would decrease \( t_{p} \) only moderately (e.g., Zoladz et al. 2013), while a decrease in OXPHOS activity and/or ESA intensity, related to, for example, muscular deconditioning, enzyme deficiencies, or mitochondrial diseases, can lengthen \( t_{p} \) very significantly. This theoretical prediction confirms experimental data from single frog muscle cells over a wide range of OXPHOS capacities (Wüst et al. 2013) and from endurance-trained humans (Jones and Koppo 2005) or humans with metabolic mitochondrial myopathies (Grassi et al. 2009).

Mitochondrial volume (density) is about two- to three-fold different between untrained individuals with pulmonary VO2max of ~30 mL min\(^{-1}\) kg\(^{-1}\) (Larsen et al. 2012), average young healthy individuals within the reference range used for this study (40–50 mL min\(^{-1}\) kg\(^{-1}\); Hoppeler et al. 1973), and after endurance training or in elite athletes with pulmonary VO2max 70–80 mL min\(^{-1}\) kg\(^{-1}\) (Larsen et al. 2012). Zoladz et al. (2013) showed, based on muscle biopsy of the vastus lateralis, that moderate-intensity endurance training (85% of the total training performed below the LT, in this case ~50% VO2max, and only 15% at midway between the lactate threshold and VO2max), lasting 5 weeks (four sessions of cycling per week; 40 min per session), significantly accelerates the primary phase of the pulmonary VO2 on-kinetics (by about 25%) in humans in the absence of any changes in the markers of mitochondrial biogenesis such as the level of peroxisome proliferator-activated receptor \( \gamma \) coactivator 1 (PGC1 \( \gamma \)), mitochondrial DNA copy number, cytochrome \( \epsilon \), and cytochrome oxidase subunit I contents. This presupposes that the alterations in the muscle biopsy reflect the action of the endurance training on the activated muscle during moderate-intensity cycling. If it does, it would support the postulate, based on computer simulations, that the training-induced acceleration of the VO2 on-kinetics observed during moderate-intensity cycling, accompanied by no signs of elevated OXPHOS activity, could be explained by a training-induced intensification of ESA (Zoladz et al. 2013). In other words, physical training first induces the increase in ESA intensity (\( A_{\text{ESA}} \)), which is followed by mitochondrial biogenesis (providing an increase in OXPHOS activity, \( k_{\text{OX}} \)). Similarly, prolonged moderate-intensity endurance training, lasting 20 weeks (four sessions per week; 40 min per session), which speeded phase 2 pulmonary VO2...
on-kinetics by about 19% was accompanied by no significant changes in the maximal COX activity in the muscle vastus lateralis (Zoladz et al. 2014). It was concluded based on the theoretical studies described therein (Zoladz et al. 2014) that the most likely explanation was a training-induced intensification of each-step parallel activation (ESA) of OXPHOS that occurred before elevated mitochondrial biogenesis.

Some studies have postulated a significant role of O₂ delivery (Murias et al. 2014) and/or of the uniformity of matching between intramuscular O₂ delivery and O₂ utilization (Koga et al. 2014) in determining the rate of VO₂ on-kinetics. However, several experimental studies in animal models and humans have failed to show a limitation of oxygen transport to muscle for VO₂ on-kinetics during transition to exercise, at least during exercise of moderate intensity (Grassi et al. 1998; Bangsbo et al. 2000; Grassi 2005; Nyberg et al. 2014; Richardson et al. 2015).

In our study, the rate of the muscle VO₂ on-kinetics during rest-to-work transition is expressed by the time to reach 63% of the VO₂ amplitude (t₀.₆₃). This parameter does not require the VO₂ on-kinetics to be strictly exponential, although the simulated VO₂ on-kinetics is near exponential (Fig. 4). It was argued previously (Korzeniewski and Zoladz 2006) that the behavior of the muscle VO₂ time course during the rest-to-work transition is not perfectly exponential, as it is slightly faster at the very beginning of exercise due to ESA. Such a very fast increase in VO₂ during first seconds of exercise can be seen in some animal (Zoladz et al. 2008; Wüst et al. 2011) and human studies (Chung et al. 2005), which confirms the ESA hypothesis. However, this effect is complex to interpret in pulmonary VO₂ because of the action of the phase 1 on-kinetics kinetics, which is manifest at the lung and not at the muscle. On the other hand, Hogan (2001) encountered a lag in the fall of intracellular PO₂ after the onset of contractions in single Xenopus muscle fibers, suggesting a lag in the VO₂ increase. It has been demonstrated in simulation and by direct experiment that the phase 2 pulmonary VO₂ time course also deviates from a “pure” exponential response, albeit very subtly (Benson et al. 2013, 2017). Therefore, the muscle t₀.₆₃ variable reported here is very closely related, but not strictly identical, to the frequently used parameter describing the pulmonary VO₂ on-kinetics, called time constant of the phase 2 (or primary phase) of this kinetics (τₚ) (Barstow and Molé 1991; Scheuermann et al. 2001; Whipp and Rossiter 2005). Pulmonary VO₂ kinetics can deviate significantly from the muscle VO₂ kinetic response due to the influence of the dynamics of the circulation and the intervening venous blood and pulmonary gas volumes between the muscle cell and the pulmonary VO₂ measurement (Benson et al. 2013).

However, it should be stressed that any difference between t₀.₆₃ and τ of muscle VO₂ in our simulations is disappearedly small and of little functional importance. Muscle t₀.₆₃ is nearly linearly proportional to ΔPCr between work and rest, as demonstrated in Figure 1. This is in agreement with the results of the experiments by Barstow et al. (1994) and Phillips et al. (1995). Therefore, the rate of the VO₂ on-kinetics reflects the magnitude of disturbances in muscle metabolic stability during rest-to-work transition (compare also Fig. 4). The extremely fast pulmonary VO₂ on-kinetics (τₚ < 10 s) observed in top class endurance athletes (Barstow and Molé 1991; Jones and Koppo 2005; Zoladz et al. 2005), reflecting very high metabolic stability (Korzeniewski and Zoladz 2004), should be considered as a sign of very high resistance to fatigue during high-intensity exercise (Murgatroyd et al. 2011; for review see Grassi et al. 2015; Keir et al., 2016).

A moderate work intensity (30–35% of the muscle VO₂max, about 40% of the pulmonary VO₂max) was used in order to avoid the slow component of the VO₂ on-kinetics. There is considerable evidence to support that O₂ delivery is not limiting at this exercise intensity. In constant-power exercise pulmonary τₚ depends little on work intensity: pulmonary τₚ increases on average by about 20% between moderate and heavy exercise (see Poole and Jones 2005; for review of over 20 experimental studies). Theoretical studies, concerning muscle τₚ (t₀.₆₃), confirmed these experimental findings (Korzeniewski 2018). Computer simulations predicted that muscle t₀.₆₃ was approximately constant for different work intensities in constant-power exercise, and decreased noticeably only at lowest work intensities.

Of course, this theoretical study and the computer model used have several limitations. Every model constitutes, at best, only an approximation and simplification of the complex reality. We cannot prove that the mechanism proposed by us is entirely responsible for the extremely fast VO₂ on-kinetics in top athletes, rather we provide a quantitative argument for a plausible explanation. The role of other factors, for instance, limitations of O₂ transport and diffusion, cannot be excluded, although they seem not to be important during moderate exercise in healthy individuals. The estimation of some variables is only approximate, for example, differences in mitochondrial volume and OXPHOS activity between extreme individuals and reference individuals. Nevertheless, these restrictions do not seem to change the general conclusions of our simulations.

Conclusions

Based on our theoretical study we postulate that the very short (<10 s) time constant (τₚ ~t₀.₆₃) of the primary
phase of the pulmonary and muscle \( \dot{V}O_2 \) on-kinetics, encountered in highly endurance-trained athletes, representing very high metabolic stability during exercise, results from the synergistic action of a high resting (without ESA) OXPHOS activity related to high mitochondrial volume (density) and high each-step activation (ESA) of OXPHOS intensity. The dependence of muscle \( \tau_p \) on OXPHOS activity and/or ESA intensity is hyperbolic. For this reason, it is difficult to further reduce \( \tau_p \) by training in well-trained individuals, while \( \tau_p \) is greatly lengthened when OXPHOS activity is compromised in mitochondrial diseases.

**Conflict of Interest**

None declared.

**References**

Bangsbo, J., P. Krstrup, J. González-Alonso, R. Boushel, and B. Saltin. 2000. Muscle oxygen kinetics at onset of intense dynamic exercise in humans. Am. J. Physiol. Regul. Integr. Comp. Physiol. 279:R899–R906.

Barstow, T. J., and P. A. Molé. 1991. Linear and nonlinear characteristics of oxygen uptake kinetics during heavy exercise. J. Appl. Physiol. 71:2099–2106.

Barstow, T. J., S. Buchthal, S. Zanconato, and D. M. Cooper. 1994. Muscle energetics and pulmonary oxygen uptake kinetics during moderate exercise. J. Appl. Physiol. 77:1742–1749.

Benson, A. P., B. Grassi, and H. B. Rossiter. 2013. A validated model of oxygen uptake and circulatory dynamic interactions at exercise onset in humans. J. Appl. Physiol. 115:743–755. https://doi.org/10.1152/japplphysiol.00184.2013.

Benson, A. P., T. S. Bowen, C. Ferguson, S. R. Murgatroyd, and H. B. Rossiter. 2017. Data collection, handling, and fitting strategies to optimize accuracy and precision of oxygen uptake kinetics estimation from breath-by-breath measurements. J. Appl. Physiol. 123:227–242. https://doi.org/10.1152/japplphysiol.00988.2016.

Chung, Y., P. A. Molé, N. Sailasuta, T. K. Tran, R. Hurd, and T. Jue. 2005. Control of respiration and bioenergetics during muscle contraction. Am. J. Physiol.-Cell Physiol. 288:C730–C738.

Grassi, B. 2005. Delayed metabolic activation of oxidative phosphorylation in skeletal muscle at exercise onset. Med. Sci. Sports Exerc. 37:1567–1573.

Grassi, B., D. C. Poole, R. S. Richardson, D. R. Knight, B. K. Erickson, and P. D. Wagner. 1996. Muscle \( \dot{O}_2 \) uptake kinetics in humans: implications for metabolic control. J. Appl. Physiol. 80:988–998.

Grassi, B., L. B. Gladden, M. Samaja, C. M. Stary, and M. C. Hogan. 1998. Faster adjustment of \( \dot{O}_2 \) delivery does not affect \( \dot{V}O_2 \) on-kinetics in isolated in situ canine muscle. J. Appl. Physiol. 85:1394–1403.

Grassi, B., S. Porcelli, M. Marzorati, F. Lanfranconi, P. Vago, C. Marconi, et al. 2009. Metabolic myopathies: functional evaluation by analysis of oxygen uptake kinetics. Med. Sci. Sports Exerc. 41:2120–2127. https://doi.org/10.1249/MSS.0b013e3181aae96b.

Grassi, B., S. Porcelli, D. Salvadori, and J. A. Zoladz. 2011. Slow \( \dot{V}O_2 \) kinetics during moderate-intensity exercise as markers of lower metabolic stability and lower exercise tolerance. Eur. J. Appl. Physiol. 111:345–355. https://doi.org/10.1007/s00421-010-1609-1.

Grassi, B., H. B. Rossiter, and J. A. Zoladz. 2015. Skeletal muscle fatigue and decreased efficiency: two sides of the same coin? Exerc. Sport Sci. Rev. 43:75–83. https://doi.org/10.1249/JES.0000000000000043. Review.

Hochachka, P. 1994. Muscles as metabolic machines. CRC Press, Boca Raton.

Hogan, M. C. 2001. Fall in intracellular \( \dot{P}O_2 \) at the onset of contractions in Xenopus single skeletal muscle fibers. J. Appl. Physiol. 90:1871–1876.

Hoppeler, H., P. Lüthi, H. Claassen, E. R. Weibel, and H. Howald. 1973. The ultrastructure of the normal human skeletal muscle. A morphometric analysis on untrained men, women and well-trained orienteers. Pflügers Arch. Eur. J. Physiol. 344:217–232.

Jones, A. M., and K. Koppo. 2005. Effect of training on \( \dot{V}O_2 \) kinetics and performance. Pp. 373–397 in A. M. Jones and D. C. Poole, eds. Oxygen uptake kinetics in sport, exercise and medicine. Routledge Book Company (Taylor and Francis Group), London.

Keir, D. A., A. P. Benson, L. K. Love, T. C. Robertson, H. B. Rossiter, and J. M. Kowachuk. 2016. Influence of muscle metabolic heterogeneity in determining the \( \dot{V}O_2p \) kinetic response to ramp-incremental exercise. J. Appl. Physiol. 120:503–513.

Koga, S., H. B. Rossiter, I. Heinonen, T. I. Musch, and D. C. Poole. 2014. Dynamic heterogeneity of exercising muscle blood flow and \( \dot{O}_2 \) utilization. Med. Sci. Sports Exerc. 46:860–876. https://doi.org/10.1249/MSS.000000000000178.

Korzeniewski, B. 1998. Regulation of ATP supply during muscle contraction: theoretical studies. Biochem. J. 330:1189–1195.

Korzeniewski, B. 2007. Regulation of oxidative phosphorylation through parallel activation. Biophys. Chem. 129:93–110.

Korzeniewski, B. 2017. Regulation of oxidative phosphorylation through each-step activation (ESA): evidences from computer modeling. Prog. Biophys. Mol. Biol. 125:1–23. https://doi.org/10.1016/j.pbiomolbio.2016.12.001.

Korzeniewski, B. 2018. Regulation of oxidative phosphorylation is different in electrically- and cortically skeletal muscle. PLoS ONE 13:e0195620. https://doi.org/10.1371/journal.pone.0195620.
Korzeniewski, B., and P. Liguzinski. 2004. Theoretical studies on the regulation of anaerobic glycolysis and its influence on oxidative phosphorylation in skeletal muscle. Biophys. Chem. 110:147–169.

Korzeniewski, B., and H. B. Rossiter. 2015. Each-step activation of oxidative phosphorylation is necessary to explain muscle metabolite kinetic responses to exercise and recovery in humans. J. Physiol. 593:5255–5268. https://doi.org/10.1113/jp271299.

Korzeniewski, B., and J. A. Zoladz. 2001. A model of oxidative phosphorylation in mammalian skeletal muscle. Biophys. Chem. 92:17–34.

Korzeniewski, B., and J. A. Zoladz. 2004. Factors determining the oxygen consumption rate (\(\dot{V}O_2\)) on-kinetics in skeletal muscle. Biochem. J. 379:703–710.

Korzeniewski, B., and J. A. Zoladz. 2006. Biochemical background of the \(\dot{V}O_2\) on-kinetics in skeletal muscles. J. Physiol. Sci. 56:1–12.

Larsen, S., J. Nielsen, C. N. Hansen, L. B. Nielsen, F. Wibrand, N. Stride, et al. 2012. Biomarkers of mitochondrial content in skeletal muscle of healthy young human subjects. J. Physiol. 590:3349–3360. https://doi.org/10.1113/jphysiol.2012.230185.

Murgatroyd, S. R., C. Ferguson, S. A. Ward, B. J. Whipp, and H. B. Rossiter. 2011. Pulmonary \(\dot{V}O_2\) uptake kinetics as a determinant of high-intensity exercise tolerance in humans. J. Appl. Physiol. 110:1598–1606. https://doi.org/10.1152/japplphysiol.01092.2010.

Murias, J. M., M. D. Spencer, and D. H. Paterson. 2014. The critical role of \(O_2\) provision in the dynamic adjustment of oxidative phosphorylation. Exerc. Sport Sci. Rev. 42:4–11. https://doi.org/10.1249/ESR.0000000000000005.

Nyberg, M., P. M. Christensen, S. P. Mortensen, Y. Hellsten, and J. Bangsbo. 2014. Infusion of ATP increases leg oxygen delivery but not oxygen uptake in the initial phase of intense knee-extensor exercise in humans. Exp. Physiol. 99:1399–1408. https://doi.org/10.1113/exphysiol.2014.081141.

Phillips, S. M., H. J. Green, M. J. MacDonald, and R. L. Hughson. 1995. Progressive effect of endurance training on \(\dot{V}O_2\) kinetics at the onset of submaximal exercise. J. Appl. Physiol. 79:1914–1920.

Poole, D. C., and A. M. Jones. 2005. Towards an understanding of the mechanistic bases of \(\dot{V}O_2\) kinetics. Pp. 294–328 in A. M. Jones and D. C. Poole, eds. Oxygen uptake kinetics in sport, exercise and medicine. Routledge Book Company (Taylor and Francis Group), London.

Poole, D. C., and A. M. Jones. 2012. Oxygen uptake kinetics. Compr. Physiol. 2:933–996. https://doi.org/10.1002/cphy.c100072.

Richardson, R. S., C. Wary, D. W. Wray, J. Hoff, H. B. Rossiter, G. Layec, et al. 2015. MRS Evidence of adequate \(O_2\) supply in human skeletal muscle at the onset of exercise. Med. Sci. Sports Exerc. 47:2299–2307. https://doi.org/10.1249/mss.0000000000006675.

Rossiter, H. B. 2011. Exercise: kinetic considerations for gas exchange. Compr. Physiol. 1:203–244. https://doi.org/10.1002/cphy.c090010.

Schalcher, C., H. Rickli, M. Brehm, D. Weilenmann, E. Oechslin, W. Kiowski, et al. 2003. Prolonged oxygen uptake kinetics during low-intensity exercise are related to poor prognosis in patients with mild-to-moderate congestive heart failure. Chest 124:580–586.

Scheuermann, B. W., B. D. Hoetling, M. L. Noble, and T. J. Barstow. 2001. The slow component of \(O_2\) uptake is not accompanied by changes in muscle EMG during repeated bouts of heavy exercise in humans. J. Physiol. 531:245–256.

Whipp, B. J., and H. B. Rossiter. 2005. The kinetics of oxygen uptake. Physiological inferences from the parameters. Pp. 62–94 in A. M. Jones, D. C. Poole, eds. Oxygen uptake kinetics in sport, exercise and medicine. Routledge Book Company (Taylor and Francis Group), London.

Wüst, R. C., B. Grassi, M. C. Hogan, R. A. Howlett, L. B. Gladden, and H. B. Rossiter. 2011. Kinetic control of oxygen consumption during contractions in self-perfused skeletal muscle. J. Physiol. 589:3995–4009. https://doi.org/10.1113/jphysiol.2010.203422.

Wüst, R. C. I., W. J. van der Laarse, and H. B. Rossiter. 2013. On-off asymmetries in oxygen consumption kinetics of single Xenopus laevis skeletal muscle fibers suggest higher-order control. J. Physiol. 591:731–744. https://doi.org/10.1113/jphysiol.2012.241992.

Zoladz, J. A., Z. Szkutnik, K. Duda, J. Majerczak, and B. Korzeniewski. 2005. Preexercise metabolic alkalosis induced via bicarbonate ingestion accelerates \(\dot{V}O_2\) kinetics at the onset of a high-power-output exercise in humans. J. Appl. Physiol. 1985:895–904.

Zoladz, J. A., L. B. Gladden, M. C. Hogan, Z. Nieckarz, and B. Grassi. 2008. Progressive recruitment of muscle fibers is not necessary for the slow component of \(\dot{V}O_2\) kinetics. J. Appl. Physiol. 1985:757–780. https://doi.org/10.1152/japplphysiol.01129.2007.

Zoladz, J. A., B. Grassi, J. Majerczak, Z. Szkutnik, M. Korostyński, J. Karasiński, et al. 2013. Training-induced acceleration of \(O_2\) uptake on-kinetics precedes muscle mitochondrial biogenesis in humans. Exp. Physiol. 98:883–898. https://doi.org/10.1113/expphysiol.2012.069443.

Zoladz, J. A., B. Grassi, J. Majerczak, Z. Szkutnik, M. Korostyński, M. Grandys, et al. 2014. Mechanisms responsible for the acceleration of pulmonary \(\dot{V}O_2\) on-kinetics in humans after prolonged endurance training. Am. J. Physiol.-Regul. Integr. Comp. Physiol. 307:R1101–R1114. https://doi.org/10.1152/ajpregu.00046.2014.

Zoladz, J. A., A. Koziel, A. Woyda-Płoszycka, J. Celichowski, and W. Jarmuszkiewicz. 2016. Endurance training increases the efficiency of rat skeletal muscle mitochondria. Pflügers Arch. Eur. J. Physiol. 468:1709–1724. https://doi.org/10.1007/s00424-016-1867-9.