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Each-step activation of oxidative phosphorylation is necessary to explain muscle metabolic kinetic responses to exercise and recovery in humans

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Running title: Each-step activation of OXPHOS in human muscle during exercise transitions

Keywords: human skeletal muscle; exercise; oxygen uptake

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Key points

- The basic control mechanisms of oxidative phosphorylation (OXPHOS) and glycolysis during work transitions in human skeletal muscle are still a matter of debate.
- We used simulations of skeletal muscle bioenergetics to identify key system features that contribute to this debate, by comparing kinetic model outputs to experimental human data, including phosphocreatine (PCr), pH, pulmonary oxygen uptake and fluxes of ATP production by OXPHOS (vOX), anaerobic glycolysis and creatine kinase in moderate and severe intensity exercise transitions.
- We found that each-step activation (ESA) of particular OXPHOS complexes, NADH supply and glycolysis, and strong (third-order) glycolytic inhibition by protons, was required to reproduce observed PCr, pH, and vOX kinetics during exercise.
- A slow decay of ESA during recovery, which was slowed further following severe exercise, was necessary to reproduce experimental findings.
- Well-tested computer models offer new insight in the control of the human skeletal muscle bioenergetic system during physical exercise.
ABSTRACT

To better understand muscle bioenergetic regulation, a previously-developed model of the skeletal muscle cell bioenergetic system was used to simulate the influence of: 1) each step activation (ESA) of NADH supply (including glycolysis) and oxidative phosphorylation (OXPHOS) complexes; and 2) glycolytic inhibition by protons, on the kinetics of ATP synthesis from OXPHOS, anaerobic glycolysis and creatine kinase (CK). Simulations were fitted to previously published experimental data of ATP production fluxes and metabolite concentrations during moderate and severe intensity exercise transitions in bilateral knee-extension in humans. Overall, computer simulations agreed well with experimental results. Specifically, a large (>5-fold) direct activation of all OXPHOS complexes was required to simulate measured phosphocreatine (PCr) and OXPHOS responses to both moderate and severe intensity exercise. In addition, slow decay of ESA was required to fit PCr recovery kinetics, and the time constant of ESA decay was slower following severe (180s) than moderate (90s) exercise. Additionally, a strong inhibition of (anaerobic) glycolysis by protons (glycolytic rate inversely proportional to the cube of proton concentration) provided the best fit to the experimental pH kinetics, and may contribute to the progressive increase in oxidative ATP supply during acidifying contractions. During severe-intensity exercise an ‘additional’ ATP usage (a 27% increase at 8 min, above the initial ATP supply) was necessary to explain the observed VO₂ slow component. Thus parallel activation of ATP usage and ATP supply (ESA), and a strong inhibition of ATP supply by anaerobic glycolysis, were necessary to simulate the kinetics of muscle bioenergetics observed in humans.
Abbreviations

$A_{GL}$, relative activation of glycolysis; $A_{OX}$, relative activation of oxidative phosphorylation; $A_{UT}$, relative activation of ATP utilization; AK, adenylate kinase; CK, creatine kinase; ESA, each-step activation; KE, bilateral knee extension exercise; MAS, malate/aspartate shuttle;

Moderate glycolysis inhibition by $H^+$, first-order dependence on $H^+$; NADH, nicotinamide adenine dinucleotide; OXPHOS, oxidative phosphorylation; PCr, phosphocreatine; $P_i$, inorganic phosphate; Strong glycolysis inhibition by $H^+$, third-order dependence on $H^+$; $S_{CK}$, stoichiometry of proton production/consumption by CK; TCA, tricarboxylic acid cycle; vCK, muscle ATP production by creatine kinase; vGL, muscle ATP production by anaerobic glycolysis; vOX, muscle ATP production by oxidative phosphorylation; vUT, muscle ATP utilization (ATP hydrolysis); $\dot{V}O_2$, oxygen uptake (muscle or pulmonary).
INTRODUCTION

The basic mechanisms of the control of the skeletal muscle cell bioenergetic system, especially oxidative phosphorylation (OXPHOS), during work transitions is still a matter of debate. According to the original proposition by Chance and Williams (Chance & Williams, 1955), based on studies on isolated mitochondria, only ATP usage (actomyosin-ATPase and Ca\(^{2+}\)-ATPase) is directly activated by Ca\(^{2+}\) during rest-work transition in skeletal muscle, while the ATP-supply system, including O\(_2\)-consuming OXPHOS (\(\dot{V}O_2\)), is activated indirectly through negative feedback via increased sarcoplasmic concentration of the products of ATP hydrolysis: ADP and P\(_i\). Several theoretical models assume, explicitly or implicitly, this mechanism (see e.g., Wilson et al., 1979; Wilson, 2015; Wu et al., 2007).

Jeneson and co-workers postulated that the mechanistic \(\dot{V}O_2\)-[ADP] dependence is at least second-order (Jeneson et al. 1996). The discovery that three TCA (tricarboxylic acid) cycle dehydrogenases (pyruvate dehydrogenase, isocitrate dehydrogenase, 2-oxoglutarate dehydrogenase) are activated by Ca\(^{2+}\) ions (Denton & McCormack, 1990; Hansford, 1980) led to the postulate that the NADH-supply system is directly activated together with ATP usage. This possibility was supported by the discovery of a large stimulation of \(\dot{V}O_2\) in isolated brain mitochondria by Ca\(^{2+}\) ions acting through activation of the malate/aspartate shuttle (MAS) (Gellerich et al. 2012).

A general model of parallel activation of ATP usage and ATP supply during muscle contractions was postulated by Hochachka (1994), but this did not specify which particular enzymes/metabolic blocks in the ATP supply system were activated. It was subsequently proposed that not only ATP usage and NADH supply (including glycolysis), but also all OXPHOS complexes (complex I, complex III, complex IV, ATP synthase, ATP/ADP carrier, P\(_i\) carrier) are directly activated by some cytosolic mechanism predominantly involving cytosolic Ca\(^{2+}\) ions, calmodulin-like protein, and protein phosphorylation, during the rest-to-work transition in skeletal and heart muscle cells (Korzeniewski, 1998; Korzeniewski, 2003; Korzeniewski, 2007; Korzeniewski, 2014, Korzeniewski, 2015). This process is termed each-step-activation (ESA) (Korzeniewski, 2014). In skeletal muscle it is likely that a mixed
mechanism (MM) is manifest, in which all OXPHOS complexes are directly activated, but to a smaller extent than ATP usage, and therefore a moderate increase in [ADP] and [P_i] cooperates with ESA to bring about OXPHOS activation (Korzeniewski, 2014). In intact heart muscle in vivo there is no (or extremely small) change in metabolite concentrations during work transitions (Katz et al. 1989). Therefore, while high expression of OXPHOS components may explain, in part, the a high sensitivity of ATP supply to very small changes in muscle metabolites, a ‘perfect’ ESA, directly activating both ATP usage and OXPHOS to the same extent, has been suggested to operate in intact heart in vivo (Korzeniewski, 2014).

The possibility of the parallel activation of ATP demand and ATP supply during rest-work transition in skeletal muscle was supported by Wüst and co-workers (Wüst et al. 2011) on the basis of experimental measurement of changes of muscle VO_2 and [PCr] after the onset of electrically-stimulated contractions in the canine hind limb. Nevertheless, evidence supporting or refuting ESA has proven technically challenging in human muscle due to the complexity involved in determining, at the necessary high temporal resolution, instantaneous intramuscular metabolite concentrations, fluxes and relative activities of the various components of the bioenergetics systems during exercise.

Additionally, it has been demonstrated both in vitro (see Connet & Sahlin, 1996 for review) and in intact human skeletal muscle (Sutton et al. 1981) that cytosolic acidification inhibits (anaerobic) glycolysis. Thus, during high-intensity exercise characterised by a progressive metabolic acidosis, glycolytic inhibition may contribute to increasing the demands of ATP provision from OXPHOS.

The ESA mechanism was proposed mainly on the basis of theoretical studies carried out using a computer model of the skeletal muscle bioenergetic system developed previously (Korzeniewski, 1998; Korzeniewski & Zoladz, 2001, Korzeniewski & Liguzinski, 2004). This model includes a simple, semi-quantitative inhibition of glycolysis by protons, in which the rate of glycolysis is inversely proportional to the instantaneous H^+ concentration (the simplest possible description) (Korzeniewski & Liguziński, 2004). Recent investigation led to the proposal that the inhibition of ATP supply from anaerobic glycolysis by progressive H^+ ion
accumulation, together with a slow decrease of ATP supply by creatine kinase (CK) and an additional progressive increase in ATP demand, may contribute importantly to the progressive increase in \( \text{VO}_2 \) seen during high-intensity constant power exercise: the muscle \( \text{VO}_2 \) slow component (Korzeniewski & Zoladz, 2015). The pulmonary \( \text{VO}_2 \) slow component is generated principally within the exercising skeletal muscles (Poole et al., 1991).

While this computational model was extensively verified by comparison with various experimentally measured parameter and variable values and system properties (see e.g., Korzeniewski, 2007; Korzeniewski, 2014 for discussion), rarely has a direct comparison with biological data been made that would provide a satisfying, strictly quantitative, verification of the model and its postulates. This is mainly because the necessary in vivo measurements of several different variables during rest-work and work-rest transitions below the lactate threshold (moderate intensity) and above critical power (severe intensity), were not previously available. Recently, several different system variables, including time courses of pulmonary \( \text{VO}_2 \), PCr and pH during transitions to and from moderate and severe intensity exercise as well as the rate of ATP supply by OXPHOS (vOX), CK (vCK) and anaerobic glycolysis (vGL) after 3 and 8 min of exercise during bilateral knee extension (KE) in humans were measured (Cannon et al. 2014). These data constitute an excellent reference point for computer model validation.

In this study, therefore, we tested the previously-developed computer model of the skeletal muscle bioenergetic system (Korzeniewski, 1998; Korzeniewski & Zoladz, 2001; Korzeniewski & Liguzinski, 2004) by direct comparison of computer simulations with published experimental data for transitions to and from moderate and severe intensity KE exercise in humans (Cannon et al. 2014). We hypothesized that a high intensity of ESA is necessary to account for the measured changes in fluxes and metabolite concentrations, as well as for the shape of time courses of these variables during transitions from rest to moderate or severe exercise and back to rest. We expected that ESA decays slowly during muscle recovery after exercise and that the characteristic decay time is longer after severe exercise than after moderate exercise. Finally, we tested the hypothesis that a progressive
increase in ATP usage during exercise and a strong glycolytic inhibition by protons in severe intensity exercise could account for the relative magnitude of the $\dot{V}O_2$ and vOX slow component measured \textit{in vivo}.
**METHODS**

**Ethical approval**

The human data used in the present study for comparison with computational simulations were previously published (Cannon et al. 2014). All procedures were approved by The Biological Sciences Faculty Research Ethics Committee, University of Leeds, and the University of Liverpool Committee on Research Ethics, and complied with the latest revision of the Declaration of Helsinki. Written informed consent was obtained from all volunteers prior to their participation in the study. Further details on the experimental human data can be found in Cannon et al. (2014).

**Computer model**

The theoretical model of the skeletal muscle cell bioenergetics including anaerobic glycolysis developed by Korzeniewski and Liguzinski (2004), based on earlier models by Korzeniewski and Zoladz (2001) and Korzeniewski (1998), was used in the present study. This model comprises particular OXPHOS complexes (complex I, complex III, complex IV, ATP synthase, ATP/ADP carrier, P, carrier), anaerobic glycolysis, CK, ATP usage, NADH supply, and proton efflux and influx.

The model has been broadly validated by comparison of its predictions with experimental data and used for numerous theoretical studies (see e.g., Korzeniewski, 2007; Korzeniewski, 2011, Korzeniewski 2014 for overview). The complete model description of the skeletal muscle bioenergetic system including anaerobic glycolysis is located on the web site: http://awe.mol.uj.edu.pl/~benio/.

**Simulation procedures**

We aimed to model the on- (rest-to-work) and off- (work-to-rest) transition during exercise in human skeletal muscle during bilateral KE (Cannon et al. 2014). These published data used magnetic resonance spectroscopy of the quadriceps and pulmonary VO₂ to
measure the kinetics of muscle and whole-body bioenergetics during and following 3 and 8
min of moderate and severe intensity KE exercise in healthy young humans (n=13, one
female; age, 27±8 years (mean ± SD); height, 177±8 cm; mass, 75±12 kg).

Simulations were made for two exercise intensities: moderate-intensity exercise
below the lactate threshold and severe-intensity exercise above critical power. It should be
noted that critical power was not measured in the original study of Cannon et al. (2014).
However, based on the non-steady-state behaviour of pulmonary VO₂ and intramuscular
metabolism, the exercise intensity in Cannon et al. (2014) is here assumed to be above
critical power (Poole et al. 1988; Jones et al. 2008); variably termed very-heavy or severe
intensity exercise (see Rossiter, 2011 for discussion). Based on the experimental data we
adjusted the activity (rate constant) of ATP usage (hydrolysis, A_{ATP}) to be elevated 22 fold
during transition from rest to moderate-intensity exercise, and 47 fold during transition from
rest to severe-intensity exercise. This gave the value of muscle VO₂ equal to about 2.6
mM/min (58 ml/kg/min) after 8 min of moderate-intensity exercise and to about 6.7 mM/min
(150 ml/kg/min) after 8 min of severe-intensity exercise. Following this, model parameter
values were adjusted independently for moderate and severe exercise, in order to best fit the
experimental data for the kinetics of muscle PCr, pH, vOX, vCK, and vGL.

The activation of oxidative phosphorylation (A_{OX}; the relative increase of rate
constants of complex I, complex III, complex IV, ATP synthase, ATP/ADP carrier, P_i carrier
and NADH supply) (Korzeniewski, 1998; Korzeniewski, 2003; Korzeniewski, 2007;
Korzeniewski, 2014) was adjusted following an exponential time course at exercise onset:

\[ m_{OX} = A_{OX} - (A_{OX} - 1) \cdot e^{-t/\tau(ON_{OX})} \] (1)

where \( m_{OX} \) is the current activation (ratio of the current rate constant to the resting rate
constant) of OXPHOS, \( A_{OX} \) is the relative activation of OXPHOS during moderate and
severe-intensity exercise, \( \tau(ON_{OX}) = 3 \) s is the characteristic time of the activation of oxidative
phosphorylation (Korzeniewski, 2003) and \( t \) stands for the time after the onset of exercise.
\( \tau(ON_{OX}) \) was small enough in order not to disturb the on-transient.
It was assumed that glycolysis was directly activated \((A_{GL})\) during rest to moderate and severe intensity exercise transitions, and the magnitude of \(A_{GL}\) was adjusted to best fit the experimental data. The need for this strong direct parallel activation of glycolysis was demonstrated previously (Korzeniewski & Liguzinski, 2004). The increase in the rate constant of glycolysis was not instantaneous, but occurred exponentially:

\[
m_{GL} = A_{GL} - (A_{GL} - 1) \cdot e^{-t/\tau(ON_{GL})}
\]

(2)

where \(m_{GL}\) is the current activation (ratio of the current rate constant to the resting rate constant of glycolysis), \(A_{GL}\) is the relative activation of glycolysis during exercise, \(\tau(ON_{GL}) = 6\) s is the characteristic time for the activation of glycolysis and \(t\) stands for the time after the onset of exercise. \(\tau(ON_{GL})\) was small enough in order not to disturb the on-transient.

After termination of exercise the rate constants of ATP usage and glycolysis were decreased instantly to the initial (rest) values. The rate constants of oxidative phosphorylation complexes decreased exponentially according to the following equation (Korzeniewski, 2003):

\[
m_{OX} = 1 + (A_{OX} - 1) \cdot e^{-t/\tau(OFF_{OX})}
\]

(3)

where \(m_{OX}\) is the current activation (ratio of the current rate constant to the resting rate constant) of OXPHOS, \(A_{OX}\) is the relative direct activation of OXPHOS during exercise, \(\tau(OFF_{OX}) = 90\) s or 180 s is the characteristic decay time of the activation of oxidative phosphorylation for moderate and severe exercise, respectively, and \(t\) stands for the time after the onset of exercise.

There was no additional progressive component for ATP usage during moderate exercise. During severe exercise, in order to fit experimental data, a linear increase in ATP usage was included, beginning at 100 % of the ‘fundamental’ ATP usage rate (the rate at the immediate onset of exercise) and continuing until the end of exercise at 8 min (Paterson & Whipp, 1991; Barstow & Mole, 1991). This mechanism underlying the system behaviour of \(A_{UT}\) should not be confused with the behaviour of \(vOX\) itself (which is commonly approximated by a bi-exponential).
The influence of glycolytic inhibition by protons was interrogated using two different inhibitory states: moderate and strong. The rate of glycolysis for moderate glycolytic inhibition by protons was described by the following simple, semi-quantitative equation (as in Korzeniewski & Liguzinski, 2004):

\[ v_{GLYC} = k_{GLYC} \cdot ADP_{te} \cdot \left( \frac{H^{+}_{rest}}{H^{+}} \right) \]  

(4)

where \( k_{GLYC} \) is the rate constant of glycolysis, \( ADP_{te} \) is the cytosolic total (magnesium-bound and magnesium free) free ADP concentration, \( H^{+}_{rest} = 10^{-7} \) M (pH = 7.0) is the resting proton concentration and \( H^{+} \) is the current proton concentration.

The rate of glycolysis for strong glycolytic inhibition by protons was described by the following simple equation:

\[ v_{GLYC} = k_{GLYC} \cdot ADP_{te} \cdot \left( \frac{H^{+}_{rest}}{H^{+}} \right)^3 \]  

(5)

Therefore, it was assumed that the rate of glycolysis was inversely proportional to the cube of the current proton concentration.

Three computer simulations were carried out to identify the best-fit parameter values and conditions (\( A_{OX}, A_{GL}, \tau(OFF_{OX}), \) magnitude of ‘additional’ ATP usage, and moderate or strong inhibition of glycolysis by protons) during moderate and severe intensity exercise.

Simulation 1: **Severe** exercise with moderate glycolytic inhibition by protons.

Simulation 2: **Severe** exercise with strong glycolytic inhibition by protons.

Simulation 3: Moderate exercise with strong glycolytic inhibition by protons.

Simulations 1 and 2 first established the set of system characteristics that best fit the experimental data under conditions where fluxes, metabolite concentrations and, in particular, pH were most disturbed (severe exercise). Following this, simulation 3 was conducted using moderate exercise with the parameters of glycolytic inhibition established by the best fit from simulations 1 and 2.
The computer simulations performed in this study were compared with experimental data for transitions to and from moderate and severe intensity KE exercise in humans published previously (Cannon et al. 2014).

Figure 1 shows the results of Simulation 1. The best-fit model values with moderate glycolytic inhibition by protons (Equ. 4), were $A_{UT} = 47$, $A_{OX} = 47^{0.43}$ (5.24 fold), $A_{GL} = 47^{0.65}$ (12.21 fold), $\tau (OFF_{OX}) = 180$ s, ‘additional’ ATP usage = 27%. The simulated kinetics of PCr (% of resting value) and pH, as well as the values of ATP supply by OXPHOS ($v_{OX}$), CK ($v_{CK}$) and anaerobic glycolysis ($v_{GL}$) at 3rd and 8th min of severe exercise with moderate glycolytic inhibition by protons (Equ. 4) agreed well with the experimental data. Muscle $\dot{V}_{O_2}$, PCr, pH, ADP, $v_{OX}$, $v_{CK}$, $v_{GL}$ and ATP usage ($v_{UT}$) did not reach a steady state, but progressively changed during exercise ($\dot{V}_{O_2}$, ADP, $v_{UT}$ and $v_{OX}$ increased, while PCr, pH, $v_{CK}$ and $v_{GL}$ decreased). The main small differences between the simulation and the experimental data were a slower decrease in pH and a smaller magnitude of the initial transient alkalosis in the simulation. The former is most probably the result of insufficient glycolytic inhibition by protons (see Simulation 2 below). The latter is likely due to the stoichiometry of proton consumption and production by the CK in the Lohmann reaction. The dependence of this stoichiometry on pH was extracted from the work of Kushmerick (1997) and is $S_{CK}=0.63-(pH-6.0)*0.43$; which gives about 0.2 for pH $\sim$7.0. For a higher value of $S_{CK}$, a more pronounced initial transient alkalosis was obtained in computer simulations (not shown).

Additionally, a large slow component of the muscle $\dot{V}_{O_2}$ on kinetics appeared in this simulation (Simulation 1). It was caused mostly by the increase in ATP utilization during exercise (from 100 to 127 % of the ‘fundamental’ rate of ATP turnover), but also, to a small extent, by a moderate inhibition of ATP supply by anaerobic glycolysis ($v_{GL}$) by accumulating protons as exercise progressed, necessitating a supplementary increase in the ATP supply from OXPHOS ($v_{OX}$) (Korzeniewski & Zoladz, 2015).
The introduction of a strong glycolytic inhibition by protons (Equ. 5, glycolytic flux inversely proportional to the cube of the current proton concentration) for severe exercise, together with a stronger direct glycolytic activation at the onset of exercise (Simulation 2), significantly speeded the kinetics of the early intracellular pH response, but did not affect the pH value after 8 min of exercise. This can be seen in Fig. 2. In Simulation 2, with strong glycolytic inhibition by protons (Equ. 5), the model values were $A_{UT} = 47$, $A_{OX} = 47^{0.43}$ (5.24 fold), $A_{GL} = 47^{0.87}$ (28.49 fold), $\tau(OFF_{OX}) = 180$ s, ‘additional’ ATP usage = 27 %. This simulation gave a much better fit to the experimentally-measured time course of pH without influencing significantly the time course of PCR relative to the good fit observed in Simulation 1 (see Fig. 2). The high direct activation of (anaerobic) glycolysis led to a rapid decrease in pH after the onset of exercise, while the strong (anaerobic) glycolytic inhibition by protons that took place afterwards (slowing vGL) prevented excessive cytosolic acidification.

Generally, an excellent agreement of theoretical predictions with experimental data was observed in this simulation. Not only time courses of PCR and pH, but also the values of ATP production by OXPHOS (vOX), CK (vCK) and anaerobic glycolysis (vGL) agreed well with the experimental data. The relative increase of the slow component of the muscle $\dot{V}O_2$ on-kinetics measured between 3 and 8 minutes of exercise in this simulation (20%) was similar to that of the measured pulmonary $\dot{V}O_2$ (22 ± 8%; Cannon et al. 2014). Anaerobic glycolysis was strongly directly activated after the onset of exercise, which significantly elevated the initial ATP synthesis by this process. However, as exercise progressed, glycolysis was strongly (third-order dependence) inhibited by accumulating protons, meaning that a significant additional fraction of ATP supply was provided by oxidative phosphorylation. The additional requirement for vOX can be observed in the reduction of vGL in Fig. 2, between the peak at ~0.75 min and end-exercise (8 min). In Simulation 2 both the strong (anaerobic) glycolytic inhibition by protons and the ‘additional’ ATP usage increasing during exercise from 0 % of the ‘fundamental’ ATP usage at the onset of exercise to 27 % after 8 min of exercise contributed to the slow component of the muscle $\dot{V}O_2$ on-kinetics.
Simulation 3 (for moderate exercise and strong glycolysis inhibition by protons) also
generally agreed very well with experimental data obtained by Cannon et al. (2014). This is
shown in Fig. 3. The model values for moderate exercise with strong glycolytic inhibition by
protons (Equ. 5) were $A_{UT} = 22$, $A_{OX} = 22^{0.56}$ (5.64 fold), $A_{GL} = 22^{0.85}$ (13.84 fold), $\tau(OFF_{OX}) = 90$ s, ‘additional’ ATP usage = 0 %. As expected, both experimental and simulated fluxes and
metabolite concentrations during moderate exercise rest-to-work and work-to-rest transitions
changed much less than during severe exercise. Additionally, unlike during severe exercise,
a steady-state was achieved after ~3 minutes of exercise. The only exception seems to be
the mean experimental measurement of $v_{OX}$ after 3 min of exercise is greater than the
simulation. $v_{OX}$ is 35 % greater at 3 min than after 8 min of moderate exercise. However,
while this difference is apparently big, as shown in the original presentation (Cannon et al.
2014) it was not statistically significant (see error bars in Fig. 3). Of course, a difference in
$v_{OX}$ between 3rd and 8th min of exercise could not be reconciled with the presence of the
steady-state in the time course of the pulmonary $\dot{V}O_2$, $\dot{PCr}$ and $pH$ observed in this
experiment for moderate exercise (Cannon et al. 2014), and is likely the result of variability in
the measurement of this variable within subjects. However, it is not possible to know whether
the variability influences more the 3 min or 8 min values. Here, as in Cannon et al. (2014),
we assumed that the 3 min pulmonary $\dot{V}O_2$ value ‘overshoots’ the expected steady state,
albeit, non-significantly. Nevertheless, adjusting the simulations to give $v_{UT}$ (and $v_{OX}$) that
was intermediate between the 3rd and 8th min of moderate exercise, namely 20 mM min$^{-1}$, did
not substantially change the relative agreement between the experimental and simulated
fluxes and metabolite concentrations (simulation not shown).
DISCUSSION

The first objective of this theoretical study was to determine whether a computer model of the skeletal muscle bioenergetic system (Korzeniewski, 1998; Korzeniewski & Zoladz, 2001; Korzeniewski & Liguzinski, 2004), was able to reproduce, strictly quantitatively, a particular concrete set of experimental data. Using the data from the experiment conducted by Cannon et al. (2014) as the frame of reference (because it involved simultaneous measurements of the time courses of PCr and pH for rest-work-rest transitions during moderate and severe exercise as well as vOX, vCK and vGL after 3 and 8 min of exercise), we found that overall the computer simulations produced a very good agreement with the experimental data (Figs. 1-3). This proved that the model was able to reproduce correctly the complex set of the modelled system properties, and allowed us to test 4 specific hypotheses of the relative intensity of ESA and glycolytic inhibition by protons in contributing to the observed metabolite concentrations and fluxes during moderate and severe intensity exercise.

Specifically, the important new findings of this study were that: 1. high-intensity ESA accounted for the system behaviour (changes in fluxes and metabolite concentrations during rest-work-recovery transitions) in human skeletal muscle during severe and moderate exercise (Figs. 2 and 3, respectively); 2. the decay of ESA after exercise was slow, and slowed further by severe-intensity, compared to moderate-intensity, exercise (Figs. 2 and 3); 3. strong (third-order dependence) glycolytic inhibition by protons better simulated the time course of pH during severe exercise (Fig. 2) than moderate glycolytic inhibition (Fig. 1); 4. the relative magnitude of the VO₂ slow component in vivo was better modelled using a large additional ATP usage increasing progressively during exercise together with a strong proton inhibition of (anaerobic) glycolytic flux.

Each step activation (ESA) is obligatory for intramuscular bioenergetic flux control during exercise and recovery
This study emphasizes that an intensive ESA during exercise and slow decay of ESA after termination of exercise are obligatory to reproduce quantitatively the muscle metabolite concentrations and fluxes of experimental data. The direct activation of all OXPHOS complexes and NADH supply (A_{OX} in computer simulations) is over 5-fold, namely 5.64-fold and 5.24-fold during transition from rest to moderate and severe work, respectively. The A_{OX} is even slightly greater for moderate work, although the muscle \( \dot{V}O_2 \) is much greater for severe work, because the stability of PCr and pH is much better in the former case. \( \dot{V}O_2 \) is determined mostly by the ATP utilization rate (A_{UT}; as long as the OXPHOS capacity for ATP synthesis is not saturated in the absence of ESA, see below), while ESA intensity affects predominantly the stability of metabolite concentrations and their kinetics (Korzeniewski & Zoladz, 2004). The high predicted ESA intensity is not surprising, because the muscle \( \dot{V}O_2 \) increases 9.04 fold during the moderate rest-exercise transition, and 23.45 fold during the severe rest-exercise transition, while [ADP] increases only 2.17 fold and 5.49 fold, respectively. Therefore the phenomenological \( \dot{V}O_2 \)-[ADP] relationship is very steep, much steeper than possible from first- or even second-order rate reactions. Even a steeper phenomenological \( \dot{V}O_2 \)-[ADP] relationship is observed in some experiments (Wüst et al. 2011; Korzeniewski, 2014). For instance, it was observed that a 40-fold increase in electrically-stimulated dog muscle \( \dot{V}O_2 \) is accompanied by only 2.5-fold increase in [ADP] (Zoladz et al. 2008).

The fact that A_{OX} was smaller than A_{UT} implies that OXPHOS is directly activated in parallel with ATP usage during rest-to-work transition, but that OXPHOS activation is less than that of ATP usage. This corresponds to the mixed mechanism (MM) of bioenergetics control, where direct activation co-operates with negative-feedback activation (through an increase in [ADP] and [P_i]) in the control of OXPHOS (Korzeniewski, 2014) (see below).

Without ESA (A_{OX} = 1) the system collapses during severe exercise (A_{UT} = 47): [PCr] and [ATP] fall to zero (the latter is converted to ADP, and further, by adenylate kinase, AK, to AMP), [P_i] and [Cr] increase to maximal values, and a huge cytosolic acidification occurs while maximal muscle \( \dot{V}O_2 \) becomes limited to about 3.5 mM min^{-1}. Of course, this is only a
virtual prediction: in reality, in the absence of ESA, exercise would be slowed or terminated shortly after onset. Without ESA (\(A_{OX} = 1\)) during moderate exercise (\(A_{UT} = 22\)) and for lower ESA (say, for \(A_{OX} = 3\)) during moderate (\(A_{UT} = 22\)) and severe (\(A_{UT} = 47\)) exercise, the changes in metabolite concentrations predicted by the model are much greater than for high ESA, and therefore do not well fit the experimental data. Additionally, characteristic transition times (\(\tau\)) for VO\(_2\) and metabolite concentrations during rest-work transitions are lengthened under conditions where lower ESA activities are used (Korzeniewski & Zoladz, 2004).

Therefore, a decrease in ESA intensity in computer simulations results in wide disagreements with experimental results.

It was demonstrated previously, in the bioenergetic system of the skeletal muscle cell that, without ESA, huge changes in metabolite (ADP, PCr, P\(_i\)) concentrations take place when the relative ATP demand increases, and that the system collapses when the energy demand (rate constant of ATP usage) exceeds the relative value of about \(A_{UT} = 30\) fold above resting ATP demand. Under these conditions ATP and PCr concentrations fall to zero, Cr and P\(_i\) concentrations rise to the maximal values, [ADP] first rises to about 1500 \(\mu\)M and then decreases near zero as it is converted to AMP (by AK) and there is no further increase in muscle VO\(_2\) together with an increase of ATP demand (see Fig. 3A in Liguzinski & Korzeniewski, 2006). In the present study, the relative ATP demand (\(A_{UT}\)) for severe exercise was 47 fold greater than resting, and is therefore far beyond this ‘collapse threshold’. For this reason, in the absence of ESA, system collapse is observed in the simulations of the severe intensity exercise conditions. Strong ESA allows moderate changes in muscle metabolite concentrations and pH to take place, while VO\(_2\) can reach values far above this threshold (see Fig. 3B in Liguzinski & Korzeniewski, 2006).

Our comparison of computer simulations with experimental data predicts that a slow decay of ESA during muscle recovery after exercise takes place. The simulated time course of [PCr] during recovery can be fitted to experimental data only when a slow decay of ESA is assumed – otherwise the PCr recovery would be much slower (see Korzeniewski & Zoladz, 2014). The adjusted values of the characteristic ESA decay time \(\tau(\text{OFF}_{OX})\) were 90 s and 180
s for moderate and severe exercise, respectively. This is consistent with previous suggestions that a greater muscle metabolic strain during exercise lengthens $\tau(OFF_{OX})$, sometimes leading to a transient overshoot in PCr recovery (Korzeniewski & Zoladz, 2005). It seems logical that more intensive exercise causes greater muscle metabolic stress and strain. Therefore, in our simulations ESA was necessary not only to account for the increase in muscle $\dot{V}O_2$ and vOX, and changes in metabolites during the on-transient, but also to explain system behaviour during the off-transient.

The molecular mechanism of ESA remains in question. Glancy et al. (2013) showed that the activity of essentially all OXPHOS complexes was sensitive to Ca$^{2+}$: isolated skeletal muscle mitochondria incubated with glutamate/malate and exposed to increased Ca$^{2+}$, increased overall OXPHOS activity by about 2 fold. In electrically-stimulated canine muscle direct measurement of the $\dot{V}O_2$-[ADP] relationship suggested an A$_{OX}$ of ~3-4 fold with a $\tau(ON_{OX})$ of ~10 s (Wüst et al. 2011). The present study strongly suggests that OXPHOS complexes are activated directly over 5 fold during rest-to-work transitions in humans. In other muscles or experimental conditions this direct activation of OXPHOS can be even higher (Korzeniewski, 2014). It was proposed previously that cytosolic Ca$^{2+}$ acts in vivo through some protein analogous to calmodulin that causes protein (e.g., OXPHOS complexes) phosphorylation and that is absent in the isolated mitochondrial system (Korzeniewski, 1998; Korzeniewski, 2007; Korzeniewski, 2014).

Generally, the consequence of ESA is that the regulation of OXPHOS in intact skeletal muscle is completely different than in isolated mitochondria (at least in the absence of Ca$^{2+}$). In other words, the resting state in muscles is different from state 4 in isolated mitochondria: there is some ATP usage for ‘basal’ ATP usage by reactions that keep the cell alive (RNA/protein synthesis, ion circulation) that is responsible for about 40% of muscle $\dot{V}O_2$ in rat skeletal muscle, with the remaining $\dot{V}O_2$ due to proton leak (Rolfe & Brand, 1996). Additionally, moderate and severe exercise states in intact muscle are different from state 3 in isolated mitochondria: $\dot{V}O_2$, $\Delta p$ (protonmotive force) and NADH are much greater, while
[ADP] and [P_i] are much less in intact muscle (Korzeniewski, 2015). Therefore, experimental data from isolated mitochondria (at least in the absence of Ca^{2+}) concerning the regulation of OXPHOS during increase in energy (ATP) demand cannot be simply extrapolated to intact skeletal muscle.

During rest-to-work transitions in skeletal muscle, some moderate increase in [ADP] and [P_i] takes place and therefore the negative-feedback activation by these metabolites cooperates with ESA in the regulation of OXPHOS (the mixed mechanism; Korzeniewski, 2014). During work transitions in intact heart in vivo metabolite (PCr, P_i, ADP, ATP, NADH) concentrations are essentially constant (see e.g. Katz et al., 1989); in the nomenclature used here, this reflects a ‘pure’ ESA-controlled system. This is related to the fact that ATP supply is directly activated during low-to-high work transition to the same extent as ATP usage (discussed in detail previously; Korzeniewski et al., 2005; Korzeniewski, 2006; Korzeniewski, 2007).

While the each-step activation (ESA) mechanism was proposed previously, this study constitutes a very significant advance. Previous studies mostly used a semi-quantitative indirect validation of ESA in relation to steady-state changes in \( \dot{V}O_2 \) and [ADP] or [PCr] during rest-to-work transitions. This study, however, offers a strictly quantitative direct validation, using: 1. Several different variables (\( \dot{V}O_2 \), PCr, pH, \( v_{OX} \), \( v_{CK} \), \( v_{GL} \)); 2. Whole time courses during rest-work-recovery transitions; 3. Both moderate and severe intensity exercise. The excellent agreement of computer simulations with experimental data for such a broad range of system properties greatly supports the ESA mechanism and increases the computer model reliability.

It is in principle possible that a mechanism other than ESA could account for the discussed experimental data. On the other hand, we do not know any likely candidate for such a mechanism. Alternative proposals would have to also explain the great number of different system properties, time and intensity dependence, as explained by the ESA model with strong inhibition of glycolysis by protons, and it seems unlikely that two completely different mechanisms would be able to achieve this. We mean not only the system properties
simulated in the present study, but also numerous other properties, for instance the uniform
distribution of metabolic control among OXPHOS complexes or PCr recovery overshoot,
discussed previously (Korzeniewski, 2007; Korzeniewski, 2011; Korzeniewski 2014).

Recently Wilson (Wilson, 2015) presented a modified dynamic version of his previous
static model (Wilson et al., 1979), involving e.g., the CK system. It was successful in
reproducing semi-quantitatively some system properties, for instance the time course of PCr
after the onset of exercise. Although the kinetic description of cytochrome oxidase in this
model is very complex, the suggestion that cytochrome oxidase determines the rate of
oxygen consumption is not well supported by Metabolic Control Analysis of isolated skeletal
muscle mitochondria showing that flux control is more or less evenly distributed among
OXPHOS complexes (Rossignol et al., 1999). While this model proposes to explain the ‘lag
phase’ in pulmonary or muscle VO\(_2\) observed in several studies, it remains uncertain the
extent to which this lag reflects the mitochondria-lung or mitochondria-muscle vein delay in
oxygen transport, rather than an actual lag in mitochondrial VO\(_2\) on-kinetics. Interpretation of
thermodynamic models is complicated because they do not distinguish differing effects of
different metabolites e.g. the ADP and P\(_i\). Therefore, in computer modelling it is crucial to
validate a model for the broadest set of variable values and system properties possible, we
have done here and previously (Korzeniewski, 2007; Korzeniewski, 2011; Korzeniewski
2014; present study).

**Mechanisms of the \(\text{VO}_2\) slow component in severe intensity exercise**

Our original model of the skeletal muscle bioenergetic system with ATP and H\(^+\)
production by anaerobic glycolysis (Korzeniewski & Liguzinski, 2004) included a simple
kinetic description of glycolytic inhibition by protons. This description consists of an inverse
linear dependence of the glycolytic flux on \([\text{H}^+]\) (Equ. 4) and was used in Simulation 1 (Fig.
1). However, Simulation 2 demonstrated that the time course of pH during on-transient
measured by Cannon et al. (2014) was much better reproduced when a stronger glycolytic
inhibition by \([\text{H}^+]\) is assumed (Simulation 2, Fig. 2): specifically an inverse dependence of the
glycolytic flux on the cube of proton concentration (Equ. 5) (as well as a much stronger direct
glycolysis activation after the onset of exercise). This assumption also worked well for
moderate exercise (Simulation 3, Fig. 3). Therefore, this study provides an improved
understanding of the kinetics of control and regulation of glycolytic flux by protons.

It should be stressed that the kinetic description of the glycolytic inhibition by H$^+$ in the
model is only phenomenological, and may involve many variables, including buffers whose
relative contribution to glycolytic inhibition is intensity and/or pH dependent over the
physiologic ranges investigated, such as ammonia or inorganic phosphate. The
phenomenological proton buffering capacity is taken into account within the model. We found
that the current phenomenological model of strong glycolytic inhibition explained well both
moderate and severe intensity exercise system kinetics.

Korzeniewski & Zoladz (2015) proposed that the two main mechanisms underlying
the VO$_2$ slow component in skeletal muscle (Poole et al. 1994), at least during cycling
exercise, are the gradual inhibition of ATP supply by anaerobic glycolysis by protons
accumulating during exercise (together with a slow decay of ATP supply by CK) and a
progressive increase in ATP utilization during constant power exercise. While the latter has
been frequently supported by experimental studies (for review see e.g., Rossiter et al. 2002;
Rossiter, 2011; Poole & Jones, 2012; Cannon et al. 2014), the former proposition specifically
linking progressive inhibition of glycolysis to the VO$_2$ slow component magnitude
(Korzeniewski & Zoladz, 2015) is untested outside of computer simulations.

Here we found that the magnitude of the muscle VO$_2$ slow component in Simulations
1 and 2 was similar to the magnitude of the pulmonary VO$_2$ slow component measured by
Cannon et al. (2014) for bilateral KE exercise in humans. Specifically, the relative increase in
muscle VO$_2$ (and vOX) between 3rd and 8th min of exercise was 20 % in both simulations,
and the relative increase in pulmonary VO$_2$ was 22 % in the measured data. This suggests
that either moderate or strong glycolytic inhibition by protons likely contributes to the
dynamics of in vivo muscle energetics in general, and to the dynamics of the VO$_2$ slow
component in particular (with strong glycolytic inhibition better reproducing the dynamics of
pH), by necessitating a greater vOX as vGL becomes increasingly inhibited by proton accumulation in severe-intensity exercise in humans.

It has been proposed, on the basis of the lack of correlation between the magnitude of the pulmonary VO₂ slow component and the 'slow component' of the oxidative ATP supply (vOX), that a decrease in the P/O ratio may contribute to the muscle VO₂ on-kinetics (Cannon et al. 2014). However, the interpretation of this finding is equivocal.

First, as discussed above, because the average relative increase in pulmonary VO₂ and in vOX between 3rd and 8th min of exercise are very similar, varying P/O ratio among subjects would imply that in some subjects P/O decreases in the course of exercise, while in other subjects it increases. The latter seems unlikely. An alternative explanation could be a relatively large variability of vOX values (see error bars in Figs. 1-3) measured using the method based on the PCr recovery kinetics.

Second, as discussed in Cannon et al. (2014), there exist complexities associated with inferring muscle VO₂ kinetics from pulmonary VO₂ measurements. In this study the experimental pulmonary VO₂ (including whole-body O₂ uptake) is, naturally, much greater than the simulated muscle VO₂ (isolated to the active muscle in silico) estimated from the oxidative ATP supply (vOX). Using pulmonary gas exchange measurements to infer kinetic changes across the skeletal muscle requires a number of assumptions, including that the metabolic contribution of 'resting tissues' (basal metabolism in all bodily tissues) and 'auxiliary tissues' (activity of respiratory muscles, stabilizing muscles, cardiac muscle etc.) are constant during constant power exercise. With cycling exercise or seated single leg KE exercise, this assumption appears reasonable (e.g. Grassi et al., 1996; Krstrup et al., 2009), and thus the VO₂ slow component can be inferred to be predominantly isolated to the active locomotor muscles (Poole et al. 1991). The study of Cannon et al. (2014) used bilateral KE exercise inside a superconducting magnet to measure phosphate metabolism by 31P MRS while maximizing the muscle mass engaged in the task and simulate conditions such as in walking or cycling. In this model, the contribution of 'auxillary tissues' appears to be as much as approximately 35 to 45 % of the pulmonary VO₂ (assuming 6 or 5 kg of the mass of two
active quadriceps, respectively). Importantly it is not known whether oxygen consumption by these tissues changes substantially during exercise; any increase would contribute to the magnitude of the pulmonary VO$_2$ slow component, but not VOX or VO$_2$ in the muscles of interest.

During severe cycling exercise, working muscles are responsible for about 85% of pulmonary VO$_2$ (Poole et al. 1992), while ATP supply by ‘auxiliary tissues’ is about 8% (as estimated by Liguzinski & Korzeniewski, 2007), with the remainder being due to metabolism in ‘resting tissues’. It seems that this relative contribution is much smaller in severe KE exercise, where the muscle mass activated for the external KE power production is smaller than in running or cycling. Additionally, the only known process that could decrease P/O, namely proton leak through the inner mitochondrial membrane, is estimated to be responsible for only about 1% of muscle VO$_2$ during severe exercise (Korzeniewski & Zoladz, 2015), reducing the likelihood that mitochondrial uncoupling contributes substantially to the muscle VO$_2$ slow component magnitude. Finally, a significant (about 20% at rest) fraction of O$_2$ is consumed in the skeletal muscle cell by non-mitochondrial processes (residual oxygen consumption by e.g. NAPDH oxidase, nitric oxide synthase, or xanthine oxidase), and not by OXPHOS in mitochondria (Rolfe et al. 1999). However, it cannot be excluded currently that a decrease in P/O contributes to some extent to the muscle VO$_2$ slow component on transition to KE exercise.

We did not model the experimentally-measured pulmonary VO$_2$ (Cannon et al. 2014), because, as discussed above, the ratio of ‘active’ to ‘auxillary’ and ‘resting’ tissues VO$_2$ is unknown and may vary during exercise, and the outcome of the simulation rests entirely on the ratio selected. Additionally, it is likely that a slow decay of VO$_2$ by ‘auxiliary tissues’ and the contribution of circulatory dynamics to O$_2$ transport may dissociate pulmonary VO$_2$ kinetics from the muscle (Barstow et al., 1990; Benson et al. 2013; Korzeniewski & Zoladz, 2013), especially during recovery (Krustrup et al. 2009). Instead, for comparison with simulated data, we relied on the on-transition VOX measured from $^{31}$P MRS in Cannon et al.
(2014), but we have no independent estimation of vOX in recovery other than that inferred from [PCr] recovery kinetics.

**Data variability**

We adjusted our model by parameter fitting to averaged data presented in Cannon et al. (2014). However, there is, naturally, some variability in the original data, which may derive either from real differences between individuals (“individual variability”) or from variability inherent in the measurement methods (“method variability”). The variability of most variables in Cannon et al. (2014) is moderate, reflecting mostly “individual variability”. The only exception is vOX, which was especially variable during moderate intensity exercise. vOX is determined from the initial rate of change of PCr recovery kinetics, and therefore is influenced by the magnitude of the exercise-induced PCr depletion (Rossiter et al., 2000). In this case, the “method variability” is likely the major contributor to the overall variability in moderate intensity vOX, and is within the variability expected for this method (Rossiter et al., 2000).

We investigated the potential role of “individual variability”, using slight modifications of relevant parameter values in the computational model, for instance $A_{\text{OX}}$, $A_{\text{GL}}$, $A_{\text{UT}}$ and/or OXPHOS activity (there is no reason for these values to be identical among different individuals). The “individual variability” can be due to e.g. genetic differences or physical training. It has been proposed that training may lead not only to an increase in OXPHOS activity related to mitochondrial biogenesis, but also to elevation of ESA intensity (increase of $A_{\text{OX}}$) (Korzeniewski & Zoladz, 2003; Korzeniewski & Zoladz, 2004). It was demonstrated that both effects lead to acceleration of the $\dot{V}O_2$ on-kinetics and to improvement of metabolite stability during rest-to-work transitions (Korzeniewski & Zoladz, 2003; Korzeniewski & Zoladz, 2004). Overall, we found that small modifications of relevant parameter values in the computational model could well account for the “individual variability” observed in several parameter values in moderate and severe intensity exercise. We also believe that the apparent (although not statistically significant) overshoot in vOX in moderate exercise is
predominately due to "method variability"; however a direct identification of the source of this variability remains to be determined.

Conclusions

The computer model of the skeletal muscle cell bioenergetic system developed previously (Korzeniewski, 1998; Korzeniewski & Zoladz, 2001; Korzeniewski & Liguzinski, 2004) reproduces very well the experimental data of the time courses of [PCr] and pH during rest-work-rest transitions, as well as the ATP synthesis rate by OXPHOS (vOX), creatine kinase (vCK) and anaerobic glycolysis (vGL) after 3 and 8 min of moderate and severe bilateral knee extension (KE) exercise in humans (Cannon et al. 2014). It is demonstrated that an intensive each-step-activation (ESA) (over 5-fold direct activation of all OXPHOS complexes and NADH supply in parallel with the activation of ATP usage) was necessary to account for the changes in ATP synthesis fluxes, PCr and pH encountered in human muscles in vivo. Also a slow decay of ESA during recovery was necessary to fit experimental data. A strong inhibition of glycolysis by protons improves the agreement between the simulated and measured kinetics of pH after the onset of exercise in comparison to a moderate glycolytic inhibition. It is postulated that strong inhibition by accumulating protons of ATP supply by anaerobic glycolysis (together with a slow decay of ATP supply by CK) and an 'additional' ATP usage increasing gradually during severe exercise can explain the relative magnitude of the muscle $\dot{V}O_2$ slow component, although some contribution of a decrease in P/O cannot be explicitly excluded. Overall, this well-tested computer model provides a useful tool for studying the dynamic behaviour of muscle metabolism during exercise and recovery.
ADDITIONAL INFORMATION

Competing interests

None.

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Author contribution

B.K. and H.B.R. designed computer simulations. H.B.R. prepared experimental data for presentation. B.K. performed computer simulations and prepared figures. B.K. and H.B.R. discussed the theoretical results and wrote the manuscript. Both authors critically reviewed and approved the final version of the manuscript.

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REFERENCES


**FIGURE LEGENDS**

Fig. 1. Comparison of simulated (lines) and experimental (points) time course of PCr (% of resting values), pH and ATP supply flux during severe intensity rest-exercise-recovery with moderate glycolytic inhibition by protons (Simulation 1). Time course of simulated muscle VO₂ and ADP are also presented. Experimental points for bilateral knee extension (KE) exercise in humans were taken from Cannon et al. (2014). Vertical lines in the upper panel indicate the onset and cessation of exercise. vOX, ATP supply rate by OXPHOS; vCK, ATP supply rate by CK; vGL, ATP supply rate by anaerobic glycolysis; vUT, muscle ATP utilization (ATP hydrolysis). 0.05 was subtracted from experimental pH values in order to scale experimental pH at rest to the value of 7.0, used in computer simulations.

Fig. 2. Comparison of simulated (lines) and experimental (points) time course of PCr (% of resting values), pH and ATP supply flux during severe intensity rest-exercise-recovery transitions with strong glycolytic inhibition by protons (Simulation 2). Time course of simulated muscle VO₂ and ADP are also presented. Experimental points for bilateral knee extension (KE) exercise in humans were taken from Cannon et al. (2014). Vertical lines in the upper panel indicate the onset and cessation of exercise. vOX, ATP supply rate by OXPHOS; vCK, ATP supply rate by CK; vGL, ATP supply rate by anaerobic glycolysis; vUT, muscle ATP utilization (ATP hydrolysis). 0.05 was subtracted from experimental pH values in order to scale experimental pH at rest to the value of 7.0, used in computer simulations.

Fig. 3. Comparison of simulated (lines) and experimental (points) time course of PCr (% of resting values), pH and ATP supply flux during moderate intensity rest-exercise-recovery transitions with strong glycolytic inhibition by protons (Simulation 3). Time course of simulated muscle VO₂ and ADP are also presented. Experimental points for bilateral knee extension (KE) exercise in humans were taken from Cannon et al. (2014). Vertical lines in the upper panel indicate the onset and cessation of exercise. vOX, ATP supply rate by OXPHOS; vCK, ATP supply rate by CK; vGL, ATP supply rate by anaerobic glycolysis; vUT,
muscle ATP utilization (ATP hydrolysis). 0.08 was subtracted from experimental pH values in order to scale experimental pH at rest to the value of 7.0, used in computer simulations.