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ORIGINAL ARTICLE

P_i-based biochemical mechanism of endurance-training-induced improvement of running performance in humans

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Abstract

Purpose Endurance training improves running performance in distances where oxidative phosphorylation (OXPHOS) is the main ATP source. Here, a dynamic computer model is used to assess possible biochemical mechanisms underlying this improvement.

Methods The dynamic computer model is based on the "P_i double-threshold" mechanism of muscle fatigue, according to which the additional ATP usage appears when (1) inorganic phosphate (P_i) exceeds a critical value (Pi_{crit}); (2) exercise is terminated because of fatigue, when P_i reaches a peak value (Pi_{peak}); (3) the P_i increase and additional ATP usage increase mutually stimulate each other.

Results The endurance-training-induced increase in oxidative phosphorylation (OXPHOS) activity attenuates the reaching of Pi_{peak} by P_i (and thus of $\dot{V}O_{2max}$ by $\dot{V}O_2$) at increased power output. This in turn allows a greater work intensity, and thus higher speed, to be achieved before exercise is terminated because of fatigue at the end of the 1500 m run. Thus, identical total work is performed in a shorter time. Probably, endurance training also lowers Pi_{peak}, which improves the homeostasis of "bioenergetic" muscle metabolites: ADP, PCr, P_i and H^+ ions.

Conclusions The present dynamic computer model generates clear predictions of metabolic changes that limit performance during 1500 m running. It contributes to our mechanistic understanding of training-induced improvement in running performance and stimulates further physiological experimental studies.

Keywords Bioenergetic system · Skeletal muscle · Oxidative phosphorylation · Metabolite homeostasis · Critical power · Maximal oxygen uptake

Abbreviations

A_{UT}	Relative ATP usage activity (multiplicity of ATP usage activity at rest)
CK	Creatine kinase
CP	Critical power
ESA	Each-step activation
k_{OX}	OXPHOS activity
OXPHOS	Oxidative phosphorylation
PCr	Phosphocreatine
P_i	Inorganic phosphate
Pi_{crit}	Critical P_i, above which the additional ATP usage, underlying the slow component of the $\dot{V}O_2$ and metabolites on-kinetics, appears
Pi_{peak}	Peak P_i, at which exercise is terminated because of fatigue
PO	Power output
$t_{0.63}$	Characteristic transition time of the primary phase II of the $\dot{V}O_2$ on-kinetics (analogous to τ_p)
$\dot{V}O_2$	Oxygen uptake
$\dot{V}O_{2max}$	Maximal $\dot{V}O_2$

Introduction

It is very well known that endurance training can improve the athlete performance during various activities. For instance, it can speed up and thus shorten the time of the run on distances where oxidative ATP supply predominates,

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for example of the 1500 m run (see, e.g., Zoladz et al. 2022). However, the mechanisms responsible for this effect are not fully understood.

Endurance training can lead to an increase in total mitochondrial protein, amount/activities of enzymes involved in mitochondrial bioenergetics, skeletal muscle oxidative/respiratory capacity, OXPHOS activity in mitochondria, and mitochondrial volume density (Holloszy 1967; Baldwin et al. 1972; Krieger et al. 1980; Hoppeler et al. 1985; Wibom et al. 1992; Suter et al. 1995; Fernström et al. 2004; Tarnopolsky et al. 2007; Pesta et al. 2011; Jacobs et al. 2013; Zoladz et al. 2013, 2014, 2022; Scalzo et al. 2014).

Endurance training changes several kinetic properties of the working skeletal muscle and whole-body bioenergetic system. It increases $\dot{V}O_{2max}$, increases critical power, decreases the characteristic transition time τ_p or $t_{0.63}$ of the primary phase II of the pulmonary $\dot{V}O_2$ on-kinetics and markedly reduces the slow component of the $\dot{V}O_2$ on-kinetics (Hoppeler et al. 1985; Casaburi et al. 1987; Gaesser and Wilson 1988; Poole et al. 1990; Jenkins and Quigley 1992; Roca et al. 1992; Phillips et al. 1995; Suter et al. 1995; Womack et al. 1995; Carter et al. 2000; Fernström et al. 2004; Berger et al. 2006; Burnley and Jones 2007; Hottenrott et al. 2012; Zoladz et al. 2013; Zoladz et al. 2014).

Increased cytoplasmic P_i has a central role in muscle fatigue (Allen et al. 2008). Recently, the "P_i double-threshold" mechanism of muscle fatigue at the biochemical level was proposed (Korzeniewski and Rossiter 2020, 2021). This mechanism is based on three assumptions: (i) the additional ATP usage, which underlies the slow component of $\dot{V}O_2$ and metabolite on-kinetics, is initiated when P_i exceeds a certain critical value, termed Pi_{crit} (Korzeniewski and Rossiter 2020); (ii) muscle work is terminated because of fatigue when P_i reaches another, higher, peak value (Pi_{peak}) (Korzeniewski 2019); and (iii) P_i increase and additional ATP usage increase stimulate each other through a self-driving positive feedback mechanism (Korzeniewski and Rossiter 2020). In sufficiently intense exercise, this mechanism ultimately causes P_i to reach Pi_{peak} (and $\dot{V}O_2$ to reach $\dot{V}O_{2max}$) and leads to exercise termination because of fatigue. The first threshold corresponding to Pi_{crit} (point i), the second threshold corresponding to Pi_{peak} (point ii), and the positive feedback (point iii) were used to derive an abstract fatigue factor F representing various fatigue-related metabolites: H^+, NH_4^+, IMP, AMP, ADP, P_i etc. (Korzeniewski and Zoladz 2003).

A recently described computer model based, the "P_i double-threshold" mechanism can predict various skeletal muscle properties: changes over time of several variables including muscle $\dot{V}O_2$, cytosolic ADP, pH, PCr and P_i during rest-to-work transition in skeletal muscle; the end-exercise constancy of these variables at different power outputs above CP; the hyperbolic shape of the power-duration curve with CP as an asymptote; and the hypoxia/hyperoxia-induced decrease/increase in critical power and $\dot{V}O_{2max}$, and increase/decrease of $t_{0.63}$ (transition time of the primary phase II of the $\dot{V}O_2$ on-kinetics, analogous to τ_p) (Korzeniewski and Rossiter 2020; Korzeniewski 2023a).

Finally, the "P_i double-threshold" mechanism is able to account for training-induced changes in $\dot{V}O_{2max}$, CP and $\dot{V}O_2$ on-kinetics (shortening of $t_{0.63}$, decrease of the slow component) both in healthy individuals (Korzeniewski and Rossiter 2021; Korzeniewski 2023b) and MM patients (Korzeniewski 2022). Theoretical studies predict that muscle training causes an increase in OXPHOS activity and decrease in Pi_{peak}, the latter under the assumption that the increase in OXPHOS activity in vivo corresponds quantitatively to the increase in mitochondria volume density and/or OXPHOS (enzymes) activity in vitro.

In a recent study, Hureau et al. (2022) postulated that P_i is the main metabolite related to peripheral fatigue, while H^+ mediates in central fatigue through group III/IV muscle afferents. This finding decidedly supports the P_i double-threshold mechanism of muscle fatigue, which postulates that both working muscle and central nervous system somehow sense the metabolic state of working muscles (Korzeniewski 2019, 2023b; Korzeniewski and Rossiter 2020).

The present study is intended to test possible mechanisms at the biochemical level that are responsible for the endurance-training-induced increase of work intensity, increase in mean run speed and thus shortening of the time needed to run a certain distance. This concerns exercises where OXPHOS is the main source of ATP, in particular the 1500 m run. It is hypothesized that the endurance-training-induced marked increase in OXPHOS activity attenuates the increase in P_i during exercise of increased intensity. This in turn delays the reaching Pi_{peak} and termination of exercise. This effect is likely to be accompanied by a decrease in Pi_{peak}. It is expected that the latter effect increases the homeostasis of "bioenergetic" metabolites in the system: ADP, PCr, P_i and H^+. Nevertheless, it is also possible that a moderate increase in OXPHOS activity takes place in the result of training in the absence of any change in Pi_{peak}. Generally, it is supposed that the training-induced changes in the system cause that more intense exercise is terminated because of fatigue (P_i reaches Pi_{peak}) at the moment of completing of the 1500 m run after a shorter time than before training. Therefore, the mean ATP turnover, power output and run speed are higher, so that the time of the 1500 m run is markedly shortened.

Theoretical methods

Ethical approval

This is a purely theoretic study that did not involve any experiments on humans or animals.

Computer model

The previously developed computer model of the skeletal muscle bioenergetic system, including detailed kinetic OXPHOS description, was used (Korzeniewski 1998, 2018a, b, 2023a, b; Korzeniewski and Zoladz 2001; Korzeniewski and Liguzinski 2004; Korzeniewski and Rossiter 2015, 2020). The model involves the each-step activation (ESA) (parallel activation) mechanism, according to which ATP usage, NADH supply, glycolysis/glycogenolysis and all OXPHOS complexes are directly activated by some cytosolic factor/mechanism (likely to involve cytosolic Ca^{2+} ions and proteins (de)phosphorylation) during rest-to-work or low-to-high-work transitions in skeletal muscle, heart and other tissues (Korzeniewski 1998, 2007, 2017). The complete model description is given in Korzeniewski (2019) and located on the web site: http://bernardkorzeniewski.pl.

Within the model, the "P_i double-threshold mechanism" of muscle fatigue is expressed by a fixed $Pi_{crit} = 18$ mM, $Pi_{peak} = 25$ mM (in the standard version of the model for untrained physically active individuals) and kinetic equation for the additional ATP usage (for $P_i > Pi_{crit}$), in which the additional ATP usage flux is proportional to the current P_i-Pi_{crit} difference (Korzeniewski and Rossiter 2020; Korzeniewski 2023a). An astonishingly similar value of Pi_{crit} of 15 mM was found by Hureau et al. (2022) and related to P_i precipitation with Ca^{2+} within sarcoplasmic reticulum.

This model was widely tested and was demonstrated to be able to reproduce a broad range of apparently unrelated kinetic properties of the skeletal muscle bioenergetic system, and was used for numerous theoretical studies (see Korzeniewski 2017 for a review and Korzeniewski 2018a, b, 2019, 2021, 2022, 2023a, b; Korzeniewski and Rossiter 2020, 2021, 2022).

Computer simulations

The activity of ATP usage (A_{UT}, proportional to power output) is scaled to 1 at rest. It is increased step-wise at the onset of exercise to a desired value. One A_{UT} unit corresponds to about 3 W during whole-body exercise (e.g., cycling or running). This value may vary (between about 2–4 W), depending on, e.g., working muscle mass and type of exercise. Particular OXPHOS complexes, NADH supply block (TCA cycle, fatty acids β-oxidation, malate-aspartate shuttle) and glycolysis are activated with some delay in parallel with ATP usage at the onset of exercise through ESA (Korzeniewski 1998, 2017; Korzeniewski and Rossiter 2015).

Within the computer model, rate constants that appear in kinetic equations for all OXPHOS complexes (complex I, complex III, complex IV, ATP synthase, ATP/ADP carrier, P_i carrier) and NADH supply block (k_{C1}, k_{C3}, k_{C4}, k_{SN}, k_{EX}, k_{PI}, and k_{DH}, respectively) can be grouped into a single rate constant of OXPHOS: k_{OX}, which corresponds to OXPHOS activity. In the standard model version, corresponding to healthy physically active individuals, the relative k_{OX} is scaled to 1. This corresponds to untrained physically active individuals in the present study.

It was assumed or estimated in the present study that the OXPHOS activity increases by 20 or 12.07%, from $k_{OX} = 1$ (at $Pi_{peak} = 25.0$ mM) to $k_{OX} = 1.2$ (at $Pi_{peak} = 25.0$ or 23.745 mM) or $k_{OX} = 1.1207$ (at $Pi_{peak} = 25.0$ mM) as the result of training.

It was assumed that the total work performed during the whole the 1500 m run is identical before and after training. Within the model, this means that the product of ATP usage activity minus one ($A_{UT} - 1$) ($A_{UT} = 1$ at rest) and time of run (t_{run}) (in seconds): $(A_{UT} - 1) \times t_{run}$ is constant and equals 31,917 (a.u.). Therefore, as t_{run} decreased 1.137 times as a result of training (from 389 to 342 s) (Zoladz et al. 2022), A_{UT}-1 must increase 1.137 times. In the standard version of the model (untrained, physically active individuals) the exercise duration of 389 s corresponds (through power-duration dependence) to $A_{UT} = 83.05$. Therefore, after training $A_{UT} = (83.05 - 1) \times 1.137 + 1 = 94.33$. It was assumed that A_{UT}, corresponding to work intensity/power output, was constant throughout the whole run.

In order to obtain $t_{run} = 342$ s in the trained state for $k_{OX} = 1.2$, Pi_{peak} was decreased from 25 to 23.745 mM (t_{run} is very sensitive to parameter values and therefore exact parameter values are needed). A training-induced reduction of Pi_{peak} was already postulated previously under the assumption that the training-induced increase in OXPHOS activity in working muscles in vivo corresponds to an increase in OXPHOS enzyme activity measured in vitro (Korzeniewski and Rossiter 2021; Korzeniewski 2022). In order to check what would happen when OXPHOS activity and Pi_{peak} were not modified, but the run speed (exercise intensity) was increased, a simulation was carried out where only A_{UT} was increased from 83.05 to 94.33 (untrained state with a speeded-up run). A simulation of the trained state was also made in which OXPHOS activity was increased by 20% and A_{UT} was increased 1.137 times in relation to untrained state, but $Pi_{peak} = 25$ 0.0 mM was kept unchanged (trained state with markedly increased OXPHOS activity and unreduced Pi_{peak}). The purpose of the simulation of this fictitious

situation was to demonstrate the role of the training-induced reduction of Pi_{peak}.

Finally, a simulation of the trained state was carried out where Pi_{peak} was kept the same, as in untrained state (25.0 mM), but k_{OX} was increased by only about 12% (to 1.1207), that is much less than the rise in mitochondrial enzymes amounts/activities, especially COX (most related to OXPHOS activity, see Zoladz et al. 2022), measured in vitro (trained state with moderately increased OXPHOS activity and unchanged Pi_{peak}).

Summing up, five cases of the 1500 m run were simulated:

Case 1. Untrained state: $k_{OX} = 1$, $A_{UT} = 83.05$, $Pi_{peak} = 25.0$ mM, resulting $t_{run} = 389$ s.

Case 2. Untrained state with an increased ATP usage activity (mean run speed): $k_{OX} = 1$, $A_{UT} = 94.33$, $Pi_{peak} = 25.0$ mM, resulting t_{run} = undetermined.

Case 3. Fictitious trained state with a markedly higher OXPHOS activity and unchanged peak P_i in relation to the untrained state: $k_{OX} = 1.2$, $A_{UT} = 94.33$, $Pi_{peak} = 25.0$ mM, resulting t_{run} = undetermined.

Case 4. Trained state with a markedly higher OXPHOS activity and lowered peak P_i in relation to the untrained state: $k_{OX} = 1.2$, $A_{UT} = 94.33$, $Pi_{peak} = 23.745$ mM, resulting $t_{run} = 342$ s.

Case 5. Trained state with a moderately higher OXPHOS activity and unchanged peak P_i in relation to the untrained state: $k_{OX} = 1.1207$, $A_{UT} = 94.33$, $Pi_{peak} = 25.0$ mM, resulting $t_{run} = 342$ s.

Theoretical results

Muscle training, resulting in an increased OXPHOS activity (k_{OX}) by 20.0 or 12.1% (from 1.0 to 1.2 or 1.1207) and decreased Pi_{peak} from 25.0 to 23.745 mM or unchanged Pi_{peak} (Case 4 or 5 vs. Case 1, respectively), resulted in a modeled increase in ATP usage activity (A_{UT}) during the 1500 m run from 83.05 to 94.33 a.u. and markedly increase $\dot{V}O_{2max}$ from 13.3 mM min^{-1} to 14.8 mM min^{-1} (by 12%). This would not be possible without an increase in k_{OX} (Case 2), as exercise would be terminated because of fatigue before completing the 1500 m run. On the other hand, the training-induced increase in $\dot{V}O_{2max}$ related to rise in A_{UT} to 94.33 would be greater, to 16.0 mM min^{-1}, and the distance covered much longer than 1500 m, if the decrease in Pi_{peak} were not be associate with the k_{OX} increase by 20% (Case 3). A_{UTcrit}, corresponding to critical power, increased from 75 to 85 (by 13%), while $t_{0.63}$ decreased from 24.4 s to 20.7 s (by 16%) in trained muscle in relation to untrained muscle (Case 4 vs. Case 1). These changes are well within the ranges encountered in experimental studies. When k_{OX} was moderately increased by about 12% and Pi_{peak} remained unchanged (Case 5), the increase in $\dot{V}O_{2max}$ was very similar, as in Case 4, while the increase in CP and decrease in $t_{0.63}$ were smaller: by 11% and 11%, respectively. The theoretical results for particular cases are discussed in detail below.

After the onset of exercise in untrained muscle (Case 1), muscle $\dot{V}O_2$ first rose quickly (primary phase II of the $\dot{V}O_2$ on-kinetics) and next increased with a much lower pace (slow component of the $\dot{V}O_2$ on-kinetics). In addition, PCr, P_i and pH first changed fast (pH exhibited an initial overshoot), and then the rate of the changes fell, but were still quite marked (slow component of metabolites). ADP was increasing markedly, after the initial very fast rise, throughout the whole run period. Termination of exercise because of fatigue took place after 6.48 min (389 s) in the moment when P_i reached $Pi_{peak} = 25$ mM (and thus $\dot{V}O_2$ reached $\dot{V}O_{2max}$). This is equivalent to covering of the distance of 1500 m, as the total work produced was equivalent to $389\ s \times (83.05 - 1) = 31{,}917$ (a.u.). The slow component of the $\dot{V}O_2$ on-kinetics appeared after less than 1 min after the onset of exercise, when P_i exceeded Pi_{crit} and the additional ATP usage was initiated (Fig. 1). $t_{0.63}$ equals 24.4 s. Generally, the difference between the end-exercise and rest metabolite concentrations was relatively high (Table 1). This case represents certainly the very heavy exercise intensity domain, as defined by Whipp (1996) (see also Korzeniewski and Rossiter 2022), or severe exercise intensity domain in other classifications, as no steady state is reached and exercise is ultimately terminated because of fatigue.

When a higher mean run speed/work intensity ($A_{UT} = 94.33$) was applied without training ($k_{OX} = 1$, $Pi_{peak} = 25.0$, that is nothing was changed in the system) (Case 2), locomotory muscles became fatigued (P_i reached Pi_{peak} and $\dot{V}O_2$ reached $\dot{V}O_{2max}$) and exercise was terminated after 2.67 min (160 s) of run (Fig. 2). The total work performed was $160 \times (94.33 - 1) = 14{,}933$. This means that the run was terminated after covering the distance of $14{,}933/31917 \times 1500\ m = 702$ m. Therefore, in the absence of endurance training, an about 1.137-fold increase in work intensity (increase in mean run speed from 3.86 to 4.39 m s^{-1}) would shorten the distance covered before the termination of exercise because of fatigue over twice. Notably, $t_{0.63} = 24.1$ s was almost identical, as in Case 1.

In a fictitious case of trained muscle, where A_{UT}, proportional to work intensity/speed, was increased from 83.05 to 94.33 and OXPHOS activity k_{OX} was increased by 20% (from 1.0 to 1.2), but Pi_{peak} was unchanged in relation to untrained state (25.0 mM) (Case 3), the system remained in the very heavy exercise intensity domain. However, the exercise (run) duration of the determined intensity (mean speed) was markedly longer: $t_{run} = 12.37$ min (742 s). The total work performed was $742 \times (94.33 - 1) = 69{,}251$ (a.u.). This means that the run would be terminated after covering the distance of $69{,}251/31917 \times 1500\ m = 3256$ m, that

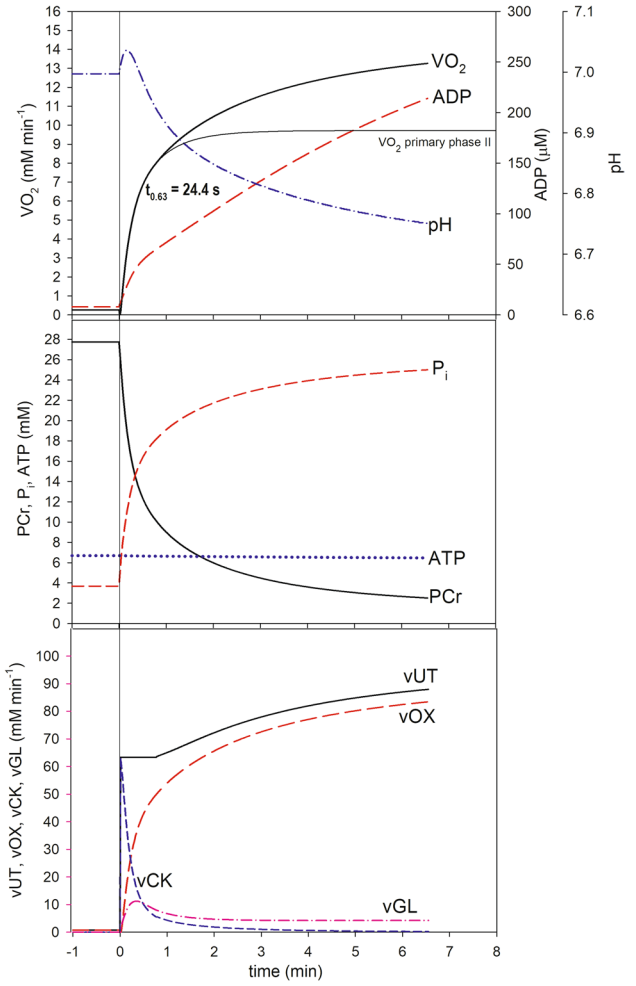

Fig. 1 Simulated time courses of selected fluxes and metabolite concentrations in working muscles during the 1500 m run of untrained individuals (Case 1). Upper panel: $\dot{V}O_2$, ADP and pH; middle panel: PCr, P_i and ATP; lower panel: ATP usage (vUT), ATP supply by OXPHOS (+aerobic glycolysis) (vOX), creatine kinase (vCK) and anaerobic glycolysis (vGL). Exercise is terminated because of muscle fatigue at the end of the run (6.55 min)

is over twice longer than 1500 m. Changes in metabolites were slower, than in untrained muscle (Case 1), but the end-exercise metabolite levels were identical, while end-exercise slow component of the $\dot{V}O_2$ on-kinetics was markedly higher. $t_{0.63} = 20.7$ s was markedly shorter, than in untrained muscle. This is demonstrated in Fig. 3, where only the first 8 min of the exercise lasting totally 12.37 min are shown.

In the realistic case of trained muscle, where OXPHOS activity increased markedly from $k_{OX} = 1.0–1.2$ and Pi_{peak} decreased from 25 to 23.745 mM in relation to rest (Case 4), the duration of the 1500 m run was shorter (342 s) than in untrained muscle (389 s), and its intensity (mean speed) was higher: $A_{UT} = 94.33$ vs. 83.05 (4.39 vs. 3.86 m s^{-1}). As a result, the total work performed before the termination of exercise because of fatigue after covering the distance of 1500 m remained unchanged in relation to untrained individuals: 342 s × (94.33 – 1) = 31,917 (a.u.). The higher work intensity and OXPHOS activity in trained than in untrained muscle resulted in a higher $\dot{V}O_2$, both total ($\dot{V}O_{2max}$) (14.83 mM min^{-1} vs. 13.26 mM min^{-1}) and the primary phase II component (11.22 mM min^{-1} vs. 9.72 mM min^{-1}). The absolute magnitude of the slow component in trained muscle in Case 4 after 342 s (end-exercise) was slightly higher, 3.61 mM min^{-1}, than in untrained muscle in Case 1 after 342 s, 3.21 mM min^{-1}, and after 389 s (end-exercise), 3.54 mM min^{-1}. $t_{0.63} = 20.7$ s was markedly shorter, than in untrained muscle in (Case 1). The metabolic homeostasis was markedly improved, that is the difference between the end-exercise and rest metabolites was markedly smaller in trained muscle in Case 4, than in untrained muscle in Case 1 (Fig. 4 and Table 1). The smaller changes in metabolites were caused by the decrease in Pi_{peak}, as other metabolites changed in parallel with P_i (Korzeniewski and Rossiter 2020, 2021, 2022).

Endurance training shifted the power–duration curve in the system in Case 4 upward in relation to Case 1 and thus increased A_{UTcrit}, corresponding to critical power (Fig. 5). Of course, only one point of this curve for untrained and trained muscle corresponds to the 1500 m run. Arrows in Fig. 5 indicate the transition from the untrained to trained state for the work intensity (A_{UT}) and duration corresponding to the 1500 m run. The lower panel in Fig. 5 demonstrates that the curvature constant W' remained essentially unaffected by training in Case 4 (the $A_{UT} - 1/t$ dependencies for untrained and trained muscle are parallel to each other). A_{UTcrit}, corresponding to critical power, increased with training from 75 to 85 (by 13%).

In the case of trained muscle, where OXPHOS activity is increased moderately by about 12% ($k_{OX} = 1.1207$),

Table 1 Simulated rest and end-run metabolite concentrations in 1500 m run in untrained (Case 1) and trained (Case 4 and 5) muscle

	Untrained (Case 1)		Trained (Case 4)		Trained (Case 5)	
Metabolite	Rest	End-run	Rest	End-run	Rest	End-run
ADP (µM)	7.9	214	7.2	159	7.5	215
PCr (mM)	27.7	2.5	28.3	6.8	28.1	2.5
P_i (mM)	3.7	25.0	3.3	23.745	3.4	25.0
pH	7.0	6.75	7.0	6.87	7.0	6.75

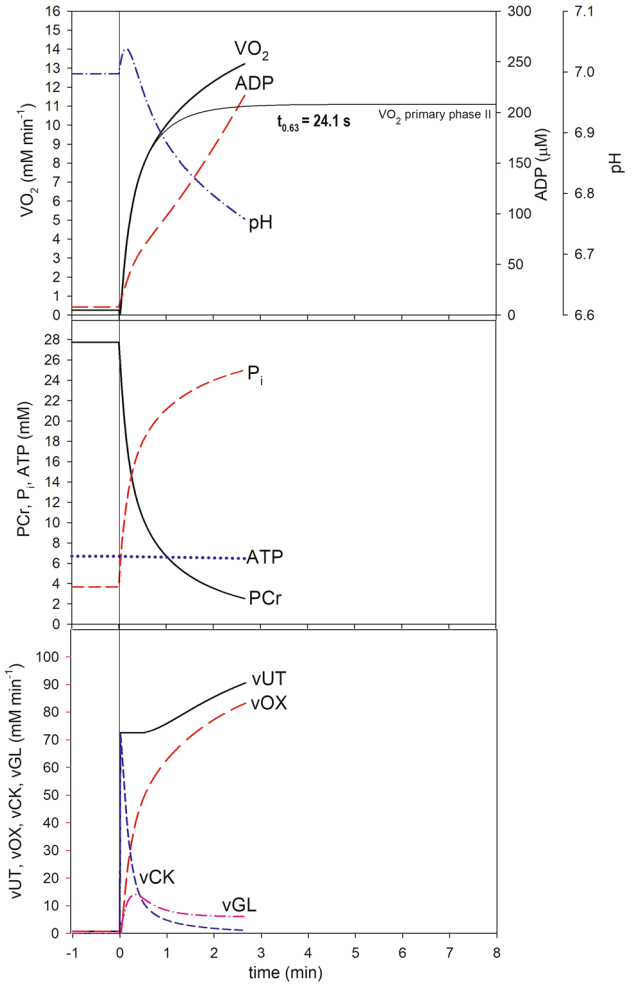

Fig. 2 Simulated time courses of selected fluxes and metabolite concentrations in working muscles during the 1500 m run of untrained individuals with increased speed (work intensity) (Case 2). Upper panel: $\dot{V}O_2$, ADP and pH; middle panel: PCr, P_i and ATP; lower panel: ATP usage (vUT), ATP supply by OXPHOS (+aerobic glycolysis) (vOX), creatine kinase (vCK) and anaerobic glycolysis (vGL). Exercise is terminated because of muscle fatigue at the end of the run (2.67 min)

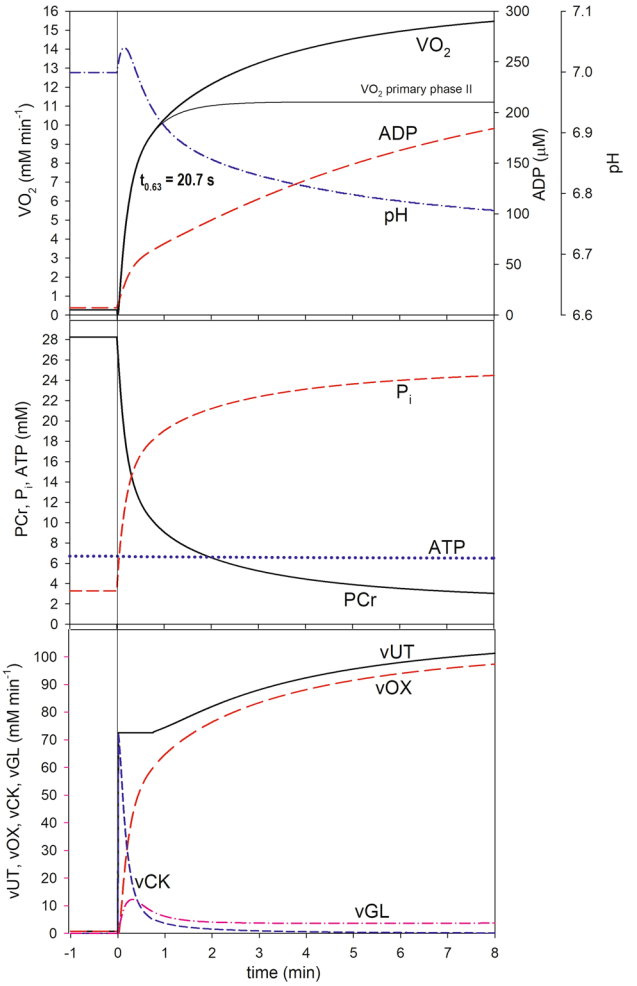

Fig. 3 Simulated time courses of selected fluxes and metabolite concentrations in working muscles during the 1500 m run of trained individuals in a fictitious case where there is no training-induced reduction in Pi_{peak} accompanying a marked OXPHOS activity increase (Case 3). Upper panel: $\dot{V}O_2$, ADP and pH; middle panel: PCr, P_i and ATP; lower panel: ATP usage (vUT), ATP supply by OXPHOS (+aerobic glycolysis) (vOX), creatine kinase (vCK) and anaerobic glycolysis (vGL). Exercise is terminated because of muscle fatigue at the end of the run after 12.36 min of simulation (only the first 8 min are shown)

and Pi_{peak} remained unchanged at 25.0 mM (Case 5), the duration of the 1500 m run was shorter (1.137 times) (342 s) than in untrained muscle (389 s), and its speed/intensity was higher (A_{UT} = 94.33 vs. 82.05), as in Case 4. The metabolic homeostasis in Case 5 was the same, as in untrained muscle (Case 1), but poorer than in Case 4 (see Table 1 and Fig. 6). This is caused by the unchanged Pi_{peak}. The power-duration curve in Case 5 was similar, as in Case 4 (not shown), whereas the training-induced increase in A_{UTcrit} (CP) in relation to rest was smaller, from 75 to 83 (by 11%).

Discussion

Mechanism of training-induced shortening of the 1500 m run time

The present study is intended to propose a P_i-based biochemical mechanism that is responsible for the endurance-training-induced increase of the mean speed of run on distances where OXPHOS is the main ATP supplier, using the 1500 m run as an example. This effect of training is associated with a greater work intensity (power output) and thus speed during run and shortening of the run time, while the

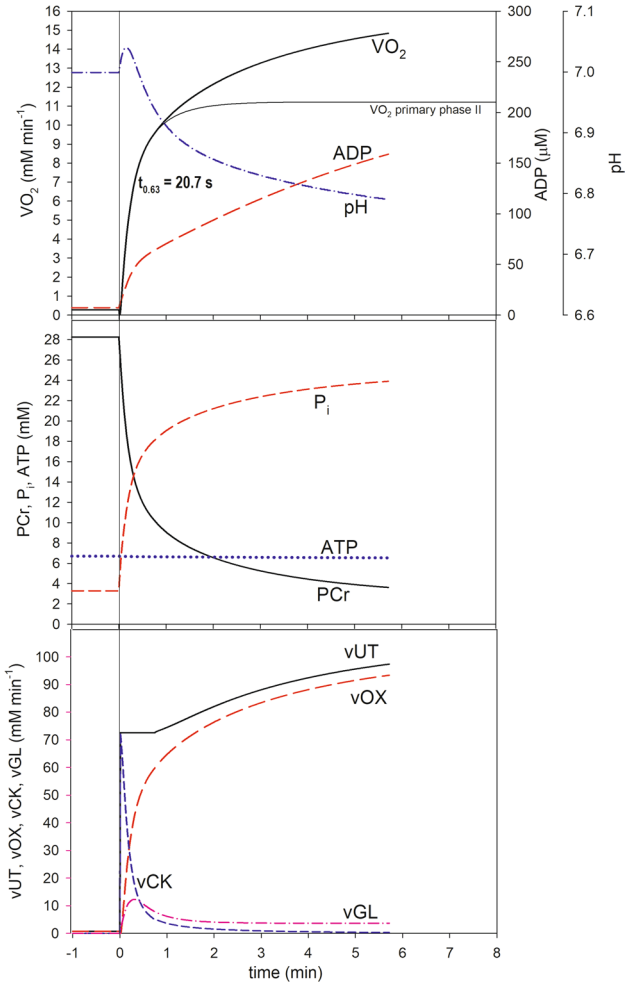

Fig. 4 Simulated time courses of selected fluxes and metabolite concentrations in working muscles during the 1500 m run of trained individuals with marked OXPHOS stimulation and lowered Pi_{peak} (Case 4). Upper panel: VO_2, ADP and pH; middle panel: PCr, P_i and ATP; lower panel: ATP usage (vUT), ATP supply by OXPHOS (+aerobic glycolysis) (vOX), creatine kinase (vCK) and anaerobic glycolysis (vGL). Exercise is terminated because of muscle fatigue at the end of the run (5.72 min)

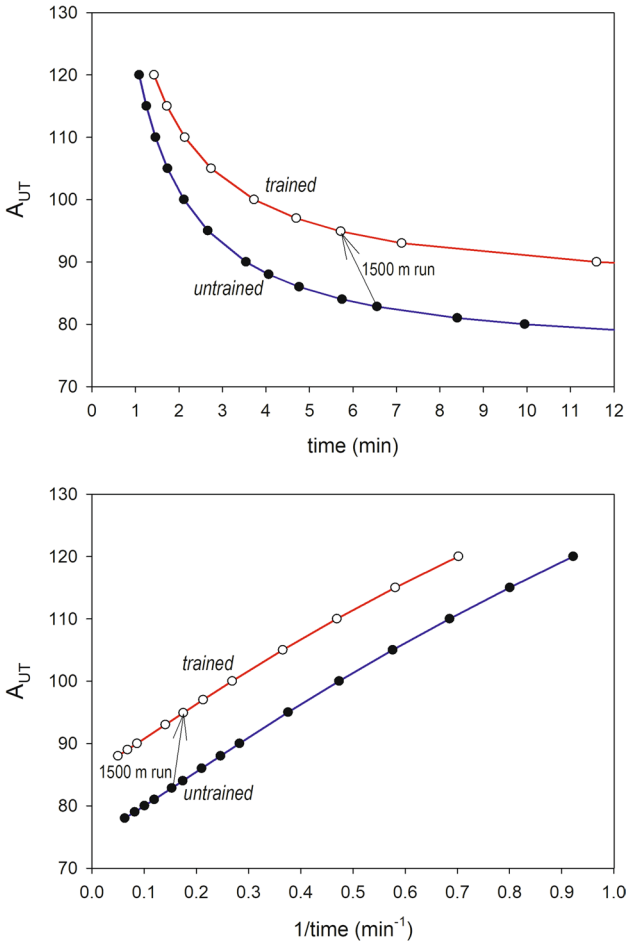

Fig. 5 Simulated power-duration dependence for the parameter values in untrained (Case 1) and trained (Case 4) muscle. Upper panel: A_{UT} (analogous to power output)-time dependence; lower panel: A_{UT}-1/time dependence

total work performed remains unchanged. In other words, the mechanism is to explain how to cause exercise termination because of fatigue at a higher work intensity just in the moment of completing of the 1500 m run, and not before (too early termination of run) or after (too slow run, too long run time). The computer model used involves the P_i double-threshold mechanism of muscle fatigue.

Computer simulations demonstrate that the encountered training-induced shortening of the 1500 m run time can be accounted for by a marked increase in OXPHOS activity (k_{OX}) and decrease of Pi_{peak} in trained vs. untrained individuals (Case 4 vs. Case 1) or by a moderate increase in OXPHOS activity at unchanged Pi_{peak} (Case 5 vs. Case 1). These possibilities can be distinguished primarily by the fact that a marked improvement of metabolite homeostasis takes place in Case 4 vs. Case 1, but not in Case 5 vs. Case 1.

General discussion

It is repeated throughout the text above that exercise is terminated because of fatigue when P_i reaches Pi_{peak}. Of course, this is a certain simplification, as people are not completely exhausted after finishing the 1500 m run and can move with a lower speed/work intensity.

Of course, the possible training-induced decrease in Pi_{peak} in itself decreases muscle performance. It was introduced in Korzeniewski and Rossiter (2021) in order to account for the training-induced increase in $\dot{V}O_{2max}$ and CP observed in experimental studies at a typical increase in OXPHOS activity measured experimentally in vitro. Therefore, either Pi_{peak} actually decreases (Case 4) or the real increase in OXPHOS activity in vivo is lower (Case 5). The advantage

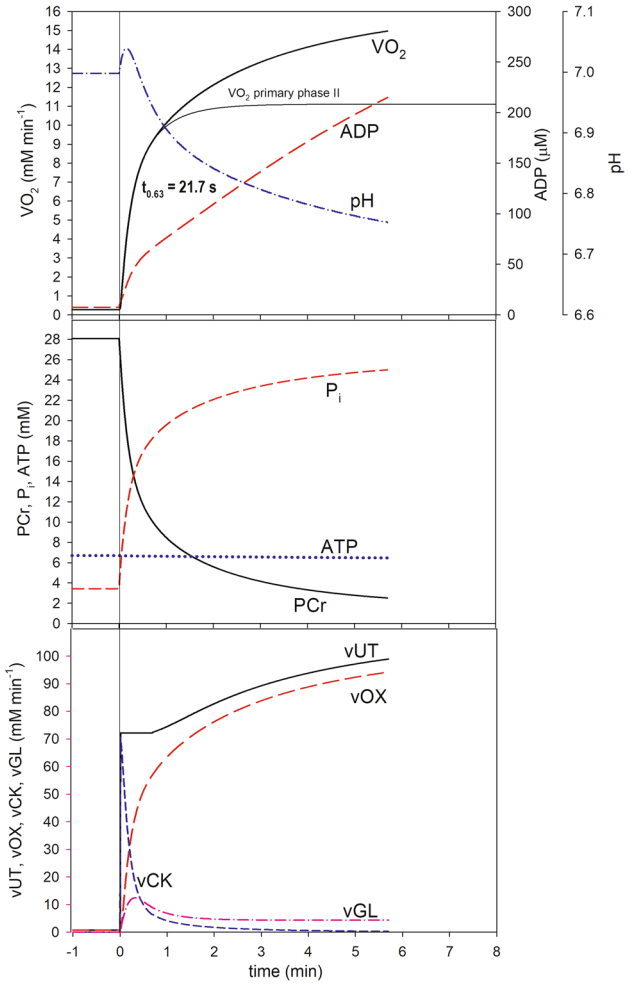

Fig. 6 Simulated time courses of selected fluxes and metabolite concentrations in working muscles during the 1500 m run of trained individuals with moderate OXPHOS activity increase and unchanged Pi_{peak} (Case 5). Upper panel: VO_2, ADP and pH; middle panel: PCr, P_i and ATP; lower panel: ATP usage (vUT), ATP supply by OXPHOS (+aerobic glycolysis) (vOX), creatine kinase (vCK) and anaerobic glycolysis (vGL). Exercise is terminated because of muscle fatigue at the end of the run (5.72 min)

of the decrease in Pi_{peak} is an improvement of metabolite homeostasis.

The parameter values used in the model, such as Pi_{peak}, Pi_{crit} or additional ATP usage rate constant (k_{add}, co-determining the magnitude of the slow component) can differ between different muscles, individuals, types of exercise, training statuses and even conditions (for instance external temperature or humidity). "Standard" values of these parameters are used in the present study, but this can be only a semi-quantitative approximation. This study is intended to provide a reliable general mechanism, and not a strictly quantitative description.

A rather high activity of the additional ATP usage is used in this study. This leads to a relatively high intensity of the $\dot{V}O_2$ and metabolites slow component. In many cases/individuals, these values can be lower.

The 1500 m run was used as an example in the present study. However, the postulated mechanism(s) of the endurance-training-induced improvement of running performance applies in principle to all run distances where OXPHOS predominates as the ATP-supplying process (apart from very long runs, e.g., marathon, where other factors, for example glycogen depletion, can contribute to muscle fatigue). In the 1500 m run, the regular (as opposed to additional) ATP usage activity, proportional to PO, is moderately above CP, so that exercise is terminated because of fatigue in the moment of reaching 1500 m. Namely, $A_{UT} = 83.05$ and 94.33 vs. $A_{UTcrit} = 75$ vs. 85 before and after training, respectively. In shorter runs, for instance the 800 m run, PO can be more above CP, as there is less time for P_i to accumulate before it reaches Pi_{peak}. On the other hand, in longer runs PO must be less above CP, as P_i increases more slowly in order not to reach Pi_{peak} before the end of the run.

Study limitations

The computer model used in the present study, as every model of this kind, constitutes only a simplification and approximation of the complex real skeletal muscle bioenergetic system it refers to.

The model is a one-compartment model, as it does not account differences between power-generating muscles (e.g., gluteus, quadriceps, biceps femoris, gastrocnemius and soleus) and various muscle fiber types within muscles. It involves parameters and variables (rate constants, activities, fluxes, and metabolite concentrations) averaged over the entire working muscles group and particular muscles. On the other hand, it is compared with "one-compartment" experimental data: muscle (or pulmonary) $\dot{V}O_2$ and muscle PCr, P_i, ADP, ATP and H^+ concentrations. When doing so, the model is able to account, at least semi-quantitatively, for a surprisingly wide range of different dynamic properties of the skeletal muscle bioenergetic system.

The "P_i double-threshold" mechanism involves explicitly only the total concentration of P_i as the main fatigue-related metabolite, which is supposed to be the most important fatigue-related factor in peripheral fatigue (Allen and Westerblad 2001; Allen et al. 2008; Hureau et al. 2022). Nevertheless, other metabolites, such as H^+, ADP, NH_4^+, IMP and AMP, can also contribute to peripheral (and central) muscle fatigue (Allen et al. 2008; Hureau et al. 2022). On the other hand, the levels of these metabolites (at least H^+ and ADP) change in parallel with P_i during exercise (Korzeniewski and Rossiter 2020, 2021). For this reason, P_i can be treated as a "representative" of the whole group of metabolites related to muscle fatigue. Some authors (Wilson et al. 1988; Sundberg

et al. 2019) proposed that deprotonated form of P_i–$H_2PO_4^-$, rather than P_i itself, is the factor that directly leads to muscle fatigue and exercise intolerance. $H_2PO_4^-$ seems an attractive candidate for the main peripheral fatigue factor as, first, its relative increase during rest-to-work transition is greater than that of P_i (Sundberg et al. 2019; Korzeniewski and Rossiter 2022) and, second, it represents both the increase in P_i and H^+ (acidification increases the fraction of P_i being in the form of $H_2PO_4^-$). Notably, a substitution within the computer model of P_i by $H_2PO_4^-$ gives similar general results (not shown). In addition, altered Ca^{2+} sensitivity and central fatigue were postulated to contribute to fatigue generation (Allen et al. 2008; Allen and Westerblad 2001). On the other hand, P_i can cause Ca^{2+} precipitation in sarcoplasmic reticulum (Allen and Westerblad 2001). In addition, as it was discussed in Korzeniewski (2019), P_i (and other related metabolites) can potentially mediate in central fatigue (the central nervous system can sense somehow the metabolic state of working muscle fibers, for instance through type III/IV afferents). Recently, Hureau et al. (2022) postulated that P_i is the main metabolite related to peripheral fatigue through precipitation with Ca^{2+} within sarcoplasmic reticulum, while H^+ mediates in central fatigue through group III/IV muscle afferents, which decidedly supports this possibility.

The model involves explicitly only muscle $\dot{V}O_2$, while pulmonary $\dot{V}O_2$ is measured in most experimental studies. A dissociation of the pulmonary and muscle $\dot{V}O_2$ kinetics can be expected under certain conditions, for instance during very intense exercise or off-transient (Poole and Jones, 2012; Krustrup et al. 2009).

The model used involves a constant capillary (extracellular) O_2. This certainly constitutes a marked simplification. For instance, the possible impact of O_2 perfusion/diffusion on the system (Wagner 2006) is not taken into account. On the other hand, O_2 concentration stabilizes during exercise on an approximately constant level (Richardson et al. 1995; McDonough et al. 2005) and muscle fiber O_2 depends little on the work rate at higher exercise intensities (Clanton et al. 2013). It was postulated by Poole and Jones (2005, 2012) that O_2 delivery is not limiting for the system under normal conditions in healthy individuals working in upright position. In addition, $t_{0.63}$, $\dot{V}O_{2max}$ and CP are little sensitive to O_2 in normoxia, hyperoxia and even mild hypoxia (Korzeniewski 2023a), and therefore, a very low capillary/mitochondrial O_2 at $\dot{V}O_{2max}$ would be required in order to markedly affect $\dot{V}O_{2max}$. Nevertheless, even in such a case, potential O_2 diffusion limitations would act through a fall in muscle fiber O_2 and acceleration of the reaching of Pi_{peak} (Korzeniewski 2023a).

In the simulations of the effect of training made in this study k_{OX} was increased by either 20 or about 12%, while Pi_{peak} was decreased from 25.0 to 23.745 or left unchanged, respectively. However, an increase in k_{OX} by, say, 25 or 15% (and appropriate decrease of Pi_{peak}) would work equally well. For this and other, already discussed reasons, the present work offers only a semi-quantitative mechanism of the training-induced speeding up and shortening of the time of the 1500 m run.

In the present computer simulations, it is assumed that: (i) the work intensity and run speed is constant during the whole run; (ii) endurance training does not change the working muscle mass; (iii) endurance training does not change the body weight; (iv) all muscles behave kinetically/metabolically in the same way during the whole run; and (v) endurance training does not change mechanical efficiency. Of course, all these assumptions certainly constitute only a rough approximations. Thus, the present computer model provides only an approximate, semi-quantitative representation of the reality. Nevertheless, this fact seems unlikely to affect markedly the general theoretical results and conclusions of the present study.

The present study is partly based on a limited set of empirical data from different studies, subjects, training protocols, etc. Some parameter values had to be adjusted. Therefore, the model predictions can be treated as only approximate and semi-quantitative. The role of in silico studies is to propose concrete mechanisms that can be verified or falsified in the experimental way and thus stimulate and direct future experimental studies.

Conclusions

The mechanism at the biochemical level responsible for the endurance-training-induced increased speed and shortening of the duration of the 1500 m run is an increase in OXPHOS activity and probably a decrease in the peak P_i value (Pi_{peak}), that is P_i concentration at which exercise is terminated because of fatigue. The latter effect improves the metabolite homeostasis during run. The greater run speed/work intensity allowing to complete the 1500 m run in a shorter time is possible because of the attenuation of P_i increase. This delays the reaching of Pi_{peak} by P_i at a given work intensity in the result of the rise in OXPHOS activity. Endurance training increases critical power and increases the whole power–duration curve, while the curvature constant W' remains essentially unchanged for the parameters values used. The present study generates semi-quantitative predictions, and thus the postulated mechanism can be verified or falsified in the experimental way. Therefore, the present study can stimulate and direct further experimental studies. The proposed general mechanism applies also to the runs on other distances, in which OXPHOS constitutes the main source of ATP. A practical conclusion of the present study is that in order to optimize the run time, the average PO during

the 1500 m run should be moderately above CP, more in shorter runs and less in longer runs.

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Declarations

Conflict of interest The author declares that there are no competing interests associated with the manuscript.

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