Mechanisms responsible for the acceleration of pulmonary $\dot{V}O_2$ on-kinetics in humans after prolonged endurance training

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Zoladz JA, Grassi B, Majerczak J, Szkutnik Z, Korostyński M, Grandyś M, Jarmuszkiewicz W, Korzeniewski B. Mechanisms responsible for the acceleration of pulmonary $\dot{V}O_2$ on-kinetics in humans after prolonged endurance training. Am J Physiol Regul Integr Comp Physiol 307: R1101–R1114, 2014. First published August 27, 2014; doi:10.1152/ajpregu.00046.2014.—The effect of prolonged endurance training on the pulmonary $\dot{V}O_2$ on- and off-kinetics in humans, in relation to muscle mitochondria biogenesis, is investigated. Eleven untrained physically active men (means ± SD: age 22.4 ± 1.5 years, $\dot{V}O_{2\text{peak}}$ 3.187 ± 0.479 ml/min) performed endurance cycling training (4 sessions per week) lasting 20 wk. Training shortened $\tau_p$ of the pulmonary $\dot{V}O_2$ on-kinetics during moderate-intensity cycling by ~19% from 28.3 ± 5.2 to 23.0 ± 4.0 s ($P = 0.005$). $\tau_p$ of the pulmonary $\dot{V}O_2$ off-kinetics decreased by ~11% from 33.7 ± 7.2 to 30.0 ± 6.6 ($P = 0.02$). Training increased (in vastus lateralis muscle) mitochondrial DNA copy number in relation to nuclear DNA (mtDNA/nDNA) (+53%) ($P = 0.014$), maximal citrate synthase (CS) activity (+38%), and CS protein content (+38%) ($P = 0.004$), whereas maximal cytochrome c oxidase (COX) activity after training tended to be only slightly (+5%) elevated ($P = 0.08$). By applying to the experimental data, our computer model of oxidative phosphorylation (OXPHOS) and using metabolic control analysis, we argue that COX activity is a much better measure of OXPHOS intensity than CS activity. According to the model, in the present study a training-induced increase in OXPHOS activity accounted for about 0–10% of the decrease in $\tau_p$ of muscle and pulmonary $\dot{V}O_2$ for the on-transient, whereas the remaining 90–100% is caused by an increase in each-step parallel activation of OXPHOS.

cycling; COX activity; CS activity; endurance training; oxygen uptake kinetics

PULMONARY $\dot{V}O_2$ on-kinetics is one of the important characteristics of the whole body and muscle energetics during physical exercise (19, 29, 56). It has been demonstrated that in “normal conditions” (i.e., normoxia, no limitations to O2 delivery, absence of pathology) the time constant of the pulmonary $\dot{V}O_2$ on-kinetics ($\tau_p$) during exercise of moderate intensity is similar to the time constant of muscle $\dot{V}O_2$ on-kinetics (18, 40). For transitions to moderate-intensity exercise most of the available experimental evidence suggests that muscle $\dot{V}O_2$ on-kinetics is mainly controlled/limited by intramuscular factors related to metabolic activation (17, 53, 56), although some recent reviews suggest a role also for O2 delivery (48) and possibly for the intramuscular matching between O2 delivery and O2 utilization (31).

It has been also reported that the muscle $\dot{V}O_2$ on- and off-kinetics studied in electrically stimulated rat muscles during contractions of low intensity (~4.5-fold increase in muscle $\dot{V}O_2$ during rest-to-work transition) are symmetrical (3). Recently Wüst et al. (63) reported that muscle $\dot{V}O_2$ on- and off-kinetics studied in high-oxidative Xenopus laevis fibers at $\dot{V}O_{2\text{max}}$ is symmetrical but in low-oxidative fibers muscle $\dot{V}O_2$ off-kinetics is more than three times slower than the muscle $\dot{V}O_2$ on-kinetics. On the other hand, Krstrup et al. (40) showed that during knee-extension exercise of moderate intensity, the mean response time of the muscle $\dot{V}O_2$ off-kinetics is by about 31% faster than the muscle $\dot{V}O_2$ on-kinetics in humans. This is in agreement with our theoretical studies involving a computer model of the bioenergetic system in skeletal muscle that suggest that the muscle $\dot{V}O_2$ off-kinetics is faster than the muscle $\dot{V}O_2$ on-kinetics (39) (our model is intended first of all to reproduce the energetic behavior of intact human skeletal muscle in situ during voluntary exercise). Far less is known about the relationship between pulmonary and muscle $\dot{V}O_2$ off-kinetics in humans. The available data show that in the case of moderate-intensity knee-extension exercise, the pulmonary $\dot{V}O_2$ off-kinetics is about twice slower than the muscle $\dot{V}O_2$ off-kinetics in humans (40).

It is well documented that as little as a few weeks of training are sufficient to decrease significantly the $\tau_p$ of the pulmonary $\dot{V}O_2$ on-kinetics (13, 20, 52, 65). The mechanism(s) underlying this training-induced acceleration, however, are not fully understood. Using a computer model of skeletal muscle bioenergetics, our group suggested (37, 38) that endurance training could accelerate muscle $\dot{V}O_2$ on-kinetics, during exercise of moderate-intensity, by an intensification of the so-called “each-step activation” (ESA) of oxidative phosphorylation (OXPHOS) (32, 33, 34) and/or by an increase in OXPHOS activity resulting from an increased mitochondrial biogenesis. It is well documented that prolonged periods of endurance training enhance mitochondrial oxidative enzymes activity (23, 25, 47) and increase mitochondrial volume density in skeletal muscle (26, 47, 59). It has been postulated that an enhanced mitochondrial biogenesis may be involved in the training-induced acceleration of the pulmonary $\dot{V}O_2$ on-kinetics in humans (20). Our computer simulations confirm that increases in OXPHOS activity would accelerate the muscle $\dot{V}O_2$ on-kinetics (37, 38). On the other hand, it has been demonstrated that in the early stage of training the acceleration of pulmonary

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VO₂ on-kinetics during moderate-intensity cycling precedes the increases of muscle mitochondrial biogenesis (52, 65), showing that at this stage of training other factor(s) is/are responsible for the training-induced acceleration of the pulmonary and muscle VO₂ on-kinetics. According to Philips et al. (52) the acceleration of pulmonary VO₂ on-kinetics observed in the early stage of training could be caused by a more rapid increase in blood flow at the onset of exercise. However, it has been shown that at least during exercise of moderate intensity the muscle VO₂ on-kinetics is substantially not limited by oxygen delivery to the muscle cells (for review see e.g., Ref. 17). Therefore, it seems that the training-induced acceleration of the pulmonary (and muscle) VO₂ on-kinetics requires some additional novel explanation.

According to Zoladz et al. (65) the training-induced acceleration of pulmonary VO₂ on-kinetics in the early stage of training is caused by intensification of ESA (32, 33, 34). This adaptive response would precede the increased mitochondrial biogenesis (65, 67). According to the ESA mechanism, an increase of ATP usage during exercise is accompanied by a simultaneous activation of oxidative phosphorylation complexes (complex I, II, III, IV, ATP synthase, ATP/ADP, P₇ carrier), probably by some cytosolic Ca²⁺-related mechanism (involving e.g., protein phosphorylation) allowing to maintain relatively stable concentrations of ATP, ADPfree, PCr, Pi, and NADH (32, 33, 34). It was postulated that during work transitions in the intact heart in vivo a perfectly balanced ESA occurs, in which ATP usage and all OXPHOS complexes are activated to the same extent, and intermediate metabolite (ADP, ATP, Pi, PCr, and NADH) concentrations remain essentially constant (34). On the other hand, in skeletal muscle a mixed mechanism would occur in which OXPHOS complexes are still directly activated by ESA, but to a smaller extent than ATP usage, and moderate (in relation to a system without ESA at the same exercise intensity) changes in intermediate metabolite concentrations take place (34). In this case the feedback activation through elevated ADPfree and P₇ would cooperate with ESA. A concept similar to ESA, named multisite modulation, was proposed in a more abstract and general way by Fell and Thomas (10) in relation to other pathways, especially glycolysis. Hochachka and coworkers (see e.g., Refs. 21 and 22) postulated that both ATP usage and ATP supply in skeletal muscle are directly activated ("simultaneous stimulation" concept), although these authors did not specify which and how many enzymes would be activated [therefore the version of parallel activation proposed by Korzeniewski (34) is called "each-step activation," ESA, to avoid confusion]. The concept of maintaining energy balance in the muscle during contractions by the mechanism of ESA recently received a significant experimental support (14, 16, 62, 65).

We argue that the widely used concept of mitochondrial biogenesis is vague and difficult to define. Mitochondrial biogenesis seems indeed a heterogeneous process with many markers [increase in mitochondrial DNA (mtDNA), citrate synthase (CS) activity, cytochrome c oxidase (COX) activity, β-hydroxyacyl-CoA dehydrogenase (β-HAD) activity, mitochondrial volume, mitochondrial membranes, etc.] (see e.g., Refs. 15, 23, 24, 26, 46, 47, 59, 60), which are related to different aspects of mitochondrial functioning that are enhanced to different extents during training. In this article we will mainly focus on one of these aspects, which is directly related to pulmonary and muscle VO₂ on- and off-kinetics, namely the increase in the OXPHOS activity.

Little is known concerning the relative contribution of the training-induced enhancement of ESA and of the increase in OXPHOS activity to the acceleration of pulmonary and muscle VO₂ on- and off-kinetics taking place after a prolonged period of training. Prolonged training is known to be associated with an enhanced mitochondrial biogenesis (23, 47, 60). We hypothesize that the acceleration of pulmonary VO₂ on- and off-kinetics after a prolonged period of endurance training would be in part due to a training-induced enhancement of ESA and in part to an increased OXPHOS activity. Therefore, in the present study we determined the effects of long-term moderate-intensity endurance training on pTp for pulmonary VO₂ on- and off-transients in moderate-intensity exercise and on "mitochondria biogenesis" markers: mtDNA, CS activity, and COX activity with special focus on the training-induced changes in the muscle COX activity. The novel aspect of the present study is that we used our computer model of skeletal muscle bioenergetics to determine quantitatively the relative contributions of OXPHOS activity and of ESA intensity to the training-induced acceleration of pulmonary VO₂ on- and off-kinetics in humans. In general terms, the most important and novel achievement of the present article is identifying which mechanism(s) is (are) responsible for the acceleration of the muscle and pulmonary VO₂ on-kinetics after a prolonged moderate-intensity training program, and to what extent.

METHODS

Subjects

Eleven untrained, but physically active, male volunteers [means ± SD: 22.4 ± 1.5 yr of age, 75.5 ± 14.2 kg body wt, 179.9 ± 8.2 cm height, 23.28 ± 3.86 kg·m⁻² body mass index (BMI), VO₂ max 3,198 ± 458 ml·min⁻¹] took part in this study after giving an informed consent. Subjects were asked to avoid heavy exercise the day before and the day of the tests, as well as to avoid caffeinated and alcoholic beverages 24 h before the tests. The study was approved by the Local Ethical Committee and was performed under the guidelines of the Declaration of Helsinki.

Exercise Protocols

All subjects performed two types of exercise protocols before and after training: an incremental exercise until exhaustion and a constant power output moderate-intensity exercise (see Incremental exercise and Constant power output test). All exercise tests were performed on a cycloergometer Ergo-Line KG 800G (Bitz, Germany) with computer-controlled power outputs. The pedaling rate during all tests was 60 rev/min.

Incremental exercise. The incremental test started with a 6-min rest, i.e., with the subject sitting on the cycloergometer, followed by a gradual increase of power output by 30 W every 3 min [until exhaustion and reaching maximal power output (POmax)] (68).

Constant power output test. The moderate-intensity constant power output test consisted of 6 min of rest with the subject sitting on the cycloergometer, followed by 6 min of cycling at 20 W, 6 min of cycling at the power output of 81 W (i.e., 90% of the lowest power output determined in individual subject), and 6 min of cycling at 20 W. In all subjects power output did not exceed 90% of the lactate threshold (LT), which for the whole group of subjects amounted to 123 ± 30 W. To improve the signal-to-noise ratio of the pulmonary VO₂ on- and off-data, the moderate-intensity exercise was repeated five times, with subsequent superposition of data. The exercise
tests were performed in 2 days, and recovery periods of at least 60 min were observed between each repetition.

**Endurance training program.** The subjects underwent a supervised endurance training program on a cycleergometer (Monark 874 E, Monark Exercise AB, Vansbro, Sweden), with sessions four times a week for 20 wk. Each training session consisted of two different exercise protocols (each lasting 40 min): a moderate-intensity continuous cycling (CC) and a high-intensity intermittent cycling (IC). CC was performed at the power output corresponding to 90% of the previously determined LT, whereas IC consisted of 6 min of unloaded cycling followed by a 3-min exercise bout at the power output corresponding to 50%Δ repeated four times and followed by 4 min of unloaded cycling. The power output corresponding to 50%Δ was calculated as 50% of the difference between the power output at which peak oxygen uptake (V\textsubscript{O}\text{2peak}) was reached and the power output at the LT [50%Δ = PO\textsubscript{LT} + 0.5 (PO\textsubscript{max} − PO\textsubscript{LT})]. To maintain an adequate training stimulus, the power output was arbitrarily adjusted after the first 5 wk of training and then after each of the 5 remaining weeks of training. From the beginning of the 6th week until the end of the 10th week, the unloaded cycling in the IC sessions was replaced by cycling at the 90% LT. During the next 5 wk, the power output corresponding to 90% LT was increased by 5% both in CC and IC protocols (from week 11 to 15) and by 15% during the last 5 wk of the training program (from week 16 to 20). The CC was performed on Tuesdays and Fridays, and IC was performed on Mondays and Thursdays. Each training session was monitored using a heart rate monitor (Polar S810, Polar Electro Oy, Kempele, Finland) and supervised by one of the authors. This endurance-training program was composed mainly of moderate-intensity cycling since ~86% of its total duration was performed below LT; i.e., at ~50% of V\textsubscript{O}\text{2peak}, and only ~14% of its total duration was performed in heavy-intensity domain at ~75% of V\textsubscript{O}\text{2peak} (for detail see Table 1). During the training period subjects were asked to keep their normal diet and to avoid taking any nutritional supplements or consuming alcohol.

**Measurements**

**Gas exchange variables and heart rate.** Gas exchange variables were recorded continuously by breath by using the Oxycon Champion, Mijnhardt (Bunnik, The Netherlands). Before each test the gas analyzers were calibrated with certified calibration gases as previously described (68). During the incremental exercise test a medium size Rudolph mask was used (dead space 90 ml), whereas during the constant power output tests the subjects were breathing via a small-sized mouthpiece (dead space 40 ml).

Heart rate was determined from the electrocardiogram tracing, registered continuously by the Hellige SMS 181 unit (Germany).

**Blood sampling.** Blood samples were collected via an Abbot Int-Catheter (18 G/1.2 x 45 mm; SL Abbott, Ireland) inserted into an antecubital vein and connected to an extension set using a “T” adapter (the length of the tube was 10 cm). Blood samples (0.5 ml each) were taken at rest (6 min before the test) and at the end of each step of the incremental test (10 s before the end of each step) as well as at the end of the incremental protocol. Samples were transferred to 1.8 ml Eppendorf tubes containing 1 mg ammonium oxalate and 5 mg sodium fluoride, mixed for about 20 s, and centrifuged.

**Plasma lactate concentration [La\textsuperscript{−}]\textsubscript{pl}.** The supernatants containing blood plasma (about 0.2 ml) were stored until minus 40°C until further analysis of lactate concentration ([La\textsuperscript{−}]\textsubscript{pl}), which was determined using an automated analyzer (Vitros 250 Dry Chemistry System, Kodak, Rochester, NY). Detection limit was 0.5 mmol/l. The LT, as previously described (68), was arbitrarily identified as the highest power output above which [La\textsuperscript{−}]\textsubscript{pl} showed a sustained increase of >0.5 mmol·l\textsuperscript{−1}·step\textsuperscript{−1}.

**Muscle biopsy.** Muscle biopsies were obtained under local anesthesia (1% lignocainhydrochloricum; Polfa Warszawa, Warsaw, Poland) from the right vastus lateralis of the quadriceps femoris muscle, ~15 cm above the upper margin of the patella, with a 2-mm diameter biopsy needle (Pro-Mag I 2.2; Angiotech, Vancouver, Canada). The specimens were frozen immediately in liquid nitrogen and used for measuring of mitochondrial DNA (mtDNA), enzymes activity, and Western immunoblotting.

**Mitochondrial DNA.** The DNA was isolated according to the manufacturer’s protocol with QIAamp DNA Mini Kit (Qiagen, Valencia, CA) and quantified by spectrophotometry using a NanoDrop ND-1000 Spectrometer (NanoDrop Technologies, Montchanin, DE). mtDNA content was measured by the quantitative PCR method using a Bio-Rad CFX 96 real-time PCR detection machine (Bio-Rad, Hercules, CA) (1). The quantity of mtDNA was corrected by simultaneous measurement of a single copy nuclear RNAse P gene Mitochondrial DNA 125 ribosomal RNA TaqMan probe (Applied Biosystems, Foster City, CA) and primers were used. The primers for real-time PCR analysis of mtDNA were designed using File Builder 3.1 and ordered as a custom TaqMan assay from Applied Biosystems. The mtDNA copy number was expressed in relation to nuclear DNA (mtDNA/nDNA). For details see Majerczak et al. (44).

**Maximal citrate synthase and cytochrome c oxidase activities.** Muscle biopsies were frozen and kept in liquid nitrogen. Sample preparation was carried out at 4°C. After weighing was completed, muscle biopsies (8–20 mg) were placed in buffer consisting of 0.32 M sucrose, 1 mM EDTA, and 10 mM Tris-HCl, pH 7.4 (SET) and homogenized with Polytron (3 x 2 s). Steps were carried out at 4°C. After a short (30 s) spin down of unbroken cells and cell debris, the supernatant was collected for determination of citrate synthase (CS) activity and cytochrome c oxidase (COX) activity. Total protein concentration of samples was determined by the Bradford method (5).

Maximal CS activity was assayed essentially as described by Freitas et al. (12). The reaction mixture (1 ml) contained 100 μM Tris, pH 8.0, 100 μM acetyl CoA, 100 mM 5,5′-di-thiobis-(2-nitrobenzoic acid) (TNB), 0.1% Triton X-100, and 25–60 μg supernatant protein (1.8–4.3 mg wet wt) and was initiated with 100 μM oxalacetate and monitored at 412 nm for 3 min at 37°C. CS activity is expressed as nmol TNB-min\textsuperscript{−1}·mg protein\textsuperscript{−1}.

Maximal COX activity was measured polarographically using a Clark-type oxygen electrode (Hansatech) in 0.7 ml of incubation medium (37°C) containing 0.17 M sucrose, 10 mM Tris-HCl (pH 7.2), 2.5 mM KH2PO4, 4 mM MgCl2, and 0.2% BSA, with 10–25 μg supernatant protein (0.72–1.8 mg wet wt). The reaction mixture

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**Table 1. Description of training load during 20 wk of endurance training program**

<table>
<thead>
<tr>
<th>Week</th>
<th>Continuous Cycling (40 min) 2 Days per Week (Tue &amp; Fri)</th>
<th>Intermittent Cycling (40 min) 2 Days per Week (Mon &amp; Thu)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1–5</td>
<td>90% LT</td>
<td>(6 min unloaded cycling + 3 min) 50% Δ Repeated 4 times each session + 4 min unloaded cycling</td>
</tr>
<tr>
<td>Week 6–10</td>
<td>90% LT</td>
<td>(6 min 90% LT + 3 min) 50% Δ Repeated 4 times each session + 4 min 90% LT</td>
</tr>
<tr>
<td>Week 11–15</td>
<td>90% LT +5%</td>
<td>(6 min 90% LT +5% + 3 min) 50% Δ Repeated 4 times each session + 4 min 90% LT +5%</td>
</tr>
<tr>
<td>Week 16–20</td>
<td>90% LT +15%</td>
<td>(6 min 90% LT +15% + 3 min) 50% Δ Repeated 4 times each session + 4 min 90% LT +15%</td>
</tr>
</tbody>
</table>

90% LT, power output corresponding to 90% of the pretraining lactate threshold; 50%Δ, difference between the power output reached at peak oxygen uptake (V\textsubscript{O}\text{2peak}) and the power output obtained at the LT [50%Δ = PO\textsubscript{LT} + 0.5 (PO\textsubscript{max} − PO\textsubscript{LT})].

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contained antimycin A (3 μg/ml), 8 mM ascorbate, 0.08% cytochrome c, and up to 2.5 mM N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD). At the end, cyanide (at 1 mM) was added. The cyanide-sensitive rates reflected COX activity (expressed in nmol O·min⁻¹·mg protein⁻¹).

**Citrate synthase content.** Protein extraction was performed using ProteoExtrac Complete Mammalian Proteome Extraction kit (Calbiochem 539779, Merck Millipore, Billerica, MA) designed to extract total proteins from mammalian tissue. Protein concentrations in skeletal muscle extracts were measured spectrophotometrically using Qubit Fluorometer (Life Technologies, Carlsbad, CA). Skeletal muscle tissue extracts were stored at −80°C until further measurements.

Skeletal muscle tissue protein extracts were mixed with sample buffer ready prepared (no. 161-0737, Bio-Rad) with addition of 2.5% 2-mercaptoethanol and denatured at 95°C for 5 min. Gels (4% stacking and 12% separating gel) were loaded with denatured extracts, which contained 11 μg of total protein. After this step, electrophoresis proteins were transferred overnight at a constant voltage (30 V) at 4°C in transfer buffer to nitrocellulose membranes (Amersham Hybond, GE Healthcare, Pittsburgh, PA). The membranes were then incubated in 5% milk in Tris-buffered saline and 0.1% Tween 20 (TBST) for 12 h. Membranes were subsequently incubated in primary antibody specific to CS (rabbit monoclonal anti-citrate synthase antibody, ab129088, Abcam, Cambridge, UK), diluted 1:5,000 in 2% BSA in TBST for 1 h, at room temperature. Membranes were washed in TBST 3 × 10 min and then incubated in secondary antibody (goat anti-rabbit ADI-SAB-300, Enzo, Life Sciences, Farmingdale, NY) diluted 1:20,000 in 2% BSA in TBST for 1 h, at room temperature. After the incubation, membranes were washed in TBST 3 × 5 min. For the detection of CS, membranes were developed with chemiluminescent detection (170-5060, ECL Western Clarity, Bio-Rad) in GeneGnome 5 (GeneSys 12.7.0, Syngene Bio Imaging, Cambridge, UK). GeneTools Syngene (Cambridge, UK) analysis software was used for densitometric analysis. The pairs of muscle samples (taken during and after the training) from each subject were run in a same gel. The molecular weight of CS was estimated by comparison with protein standards (no. 161-0377, Bio-Rad). The values of CS content were normalized to an internal standard (human vastus lateralis extract of known protein concentration), which was run on the same gel and presented in arbitrary units (AU).

**Myosin extraction and SDS-PAGE.** Muscle biopsies were mounted in Shandon cryostat with Tissue-Tek and 30–50 cryosections, 30 μm thick, were cut from each biopsy. The sections were transferred to Eppendorf tubes and myosin was extracted with 200–300 μl of lysing buffer consisting of 62.5 mM Tris, 10% glycerol, 5% 2-mercaptoethanol, 2.3% SDS, and pH 6.8. The samples were briefly vortexed and boiled for 3 min in a water bath. Myosin extracts were clarified at 13,000 g (13,000 × g) for 5 min, and supernatants were stored at minus 20°C until further use.

SDS-polyacrylamide gel electrophoresis was carried out according to Carraro and Catani (6) with 4% stacking and 6% separating gels containing 37.5% glycerol in a Mini-Protein II electrophoresis system (Bio-Rad). Myosin extracts were diluted 1:1 with sample buffer containing 0.1 M Tris-HCl (pH 6.8), 2.5% SDS, and 2.5% 2-mercaptoethanol before being boiled for 3 min. Denatured extracts, diluted 1:10 to 1:20 with lysing buffer, were loaded onto stacking gels and run at a constant voltage of 60 V for 30 min and then at 180 V for 3 h. The gels were stained with 0.1% Coomassie brilliant blue R-250 in 25% methanol and 10% acetic acid and destained with several changes of methanol-acetic acid. The pairs of muscle samples (taken before and after the training) from each subject were run in a same gel. Densitometric analysis of slow (type 1) and fast (type 2) myosin heavy chains was performed using Scion Image for Windows Beta 4.0.2 (Scion, Frederick, MD) and UVItec system equipped with COHU High Performance CCD Camera (Uvitec, Cambridge, UK). The proportions of MyHC-1 and MyHC-2 were expressed as a relative percentage of the total amount of MyHC present in biopsies from the vastus lateralis muscle.

**Modeling and estimation of pulmonary VO₂ kinetics.** The raw breath-by-breath data were preprocessed to remove occasional, artefactual values resulting from, e.g., swallowing or coughing, by means of a procedure similar to that described by Lamarra et al. (41). VO₂ responses from the bouts of repeated exercises were time aligned to the onset of the exercise and interpolated on a second-by-second basis. The resulting data were then, for each subject, superimposed, averaged over the transitions of the exercise and, finally, averaged over consecutive 10-s time intervals, as in, e.g., Rossiter et al. (57), to reduce the noise. For on-transients, t = 0 was set at the onset of exercise. For off-transients, t = 0 was set at the offset of exercise. The first cardiological phase of the on-response was not modelled. Correspondingly, the data from the first 20 s of the on-transients were ignored. Moreover, the first 20 s of the off-transients were ignored as well (for overview see e.g., Refs. 49, 56).

The on-transient VO₂ responses were modelled with a monoeponential curve

\[ V\dot{O}_2(t) = V\dot{O}_2(0) + A_1 \times \left[ 1 - e^{-\left(\frac{t-D_T}{\tau}\right)} \right], \]  

and the off-transients,VO₂ responses were modelled with a monoeponential curve

\[ V\dot{O}_2(t) = V\dot{O}_2(OFF) - A_1 \times \left[ 1 - e^{-\left(\frac{t-D_T}{\tau}\right)} \right], \]

as in Rossiter et al. (57). \( V\dot{O}_2(OFF) \) is the level attained by the on-transient curve at the end of the exercise (steady state). The off-transient curve is thus forced to start from that level, with some estimated time delay \( \delta \), and to drop down toward its asymptotic value.

The models were fitted to the corresponding data by the least-squares method using the Nonlinear Estimation module of STATISTICA, version 6. Several starting points for the iterative minimization procedure were tried to find the best fit (Fig. 1).

**Computer Simulations**

The theoretical model of skeletal muscle cell bioenergetics developed by Korzeniewski and Liguizinski (35), based on the earlier model by Korzeniewski and Zoladz (36), was used. This model comprises oxidative phosphorylation complexes, glycolysis, creatine kinase (CK), ATP usage, NADH supply, and proton efflux. The model has been broadly validated by comparison of its predictions with experimental data (see e.g., Refs. 33, 34, for discussion) and used for numerous theoretical studies (see e.g., Refs. 33 and 34 for discussion). The model was used in the present study to estimate quantitatively the effect of ESA enhancement and OXPHOS activity increase on the muscle VO₂ on- and off-kinetins. The complete description of the model is located on the web site: http://awe.mol.uj.edu.pl/~benio/.

We modeled the muscle on- (rest-to-work) and off- (work-to-rest) transition during constant moderate exercise. We assumed that the activity (rate constant) of ATP usage (hydrolysis) is instantly elevated 30 times during transition from rest to moderate exercise. This gave the value of muscle VO₂ equal to about 3.7 mM/min (~13-fold increase in muscle VO₂ during rest-to-work transition). This would be equivalent to the pulmonary VO₂ of ~1,500 ml/min, which is similar to the steady-state pulmonary VO₂ measured in the present study during moderate-intensity exercise (see Table 2). At the same time we assumed that oxidative phosphorylation (the rate constants of complex I, complex III, complex IV, ATP synthase, ATP/ADP carrier, phosphate carrier, and NADH supply are increased) is activated 30\(^{0.5} \approx 3.3\) times during rest-to-work transition (the power coefficient equal to 3.5
corresponds to moderate ESA intensity, see below). This represents the ESA mechanism discussed above. The increase in the rate constants of oxidative phosphorylation complexes was not instantaneous but occurred according to the exponential function:

\[ m = N^t - (N^t - 1) \cdot e^{-t/\tau(ON)} \]  

(3)

where \( m \) is the current activation (ratio of the current rate constant to the resting rate constant), \( N = 30 \) is the relative activation of ATP usage during exercise, the power coefficient \( x = 0.35 \) expresses the intensity of ESA, \( \tau(ON) = 3 \) s is the characteristic activation time of the activation of oxidative phosphorylation (38) (see also Ref. 62), and \( t \) stands for the time after the onset of exercise. It was assumed for simplicity that there is essentially no anaerobic glycolysis (and consequently no net H\(^+\) and lactate production) (pyruvate and NADH supply are multiplied by 1.05). This is the relative training-induced increase in CS activity which is elevated by 40% in relation to the control state, which corresponds to control state, which

\[ m = 1 + (N^t - 1) \cdot e^{-t/\tau(OFF)} \]  

(4)

where \( m \) is the current activation (ratio of the current rate constant to the resting rate constant), \( N = 30 \) is the relative activation of ATP usage during exercise, the power coefficient \( x = 0.35 \) expresses the intensity of each-step activation, \( \tau(OFF) = 100 \) s is the characteristic decay time of the activation of oxidative phosphorylation, and \( t \) stands for the time after the termination of exercise. The value of \( \tau(OFF) \) was chosen to give a realistic \( t_{0.63\text{off}} \) for PCr equal to 25–37 s depending on work intensity (see Ref. 39).

A moderate value of the ESA coefficient \( x \) (0.35 in untrained muscle) was chosen to obtain the values of \( \tau_p \) for on-transient of about 30 s, that is similar to the experimental values.

In the exponential function, \( \tau \) is the time necessary for a given variable value (for instance \( V\dot{O}_2 \) or PCr) to undergo a change equal to 63% of the final (complete) change during a given transition. Because in our simulations the transitions of muscle \( V\dot{O}_2 \) are near exponential, we use the characteristic transition time \( t_{0.63} \) instead of \( \tau_p \) as the time necessary to undergo 63% of the final (complete) change to characterize these transitions and to compare computer simulations with experimental results.

To model our experimental data and test different hypotheses concerning the mechanisms of the training-induced decrease in \( \tau_p \), we distinguished four states of the system, described by four modes of computer simulations: 1) Mode A corresponds to control state, which is untrained skeletal muscle, and constitutes a reference point for other modes. Modes B–D correspond to trained muscle with different effects on the OXPHOS system caused by training. 2) In mode B the activity of OXPHOS is elevated by 40% in relation to mode A. In other words, the rate constants of all OXPHOS complexes and NADH supply are multiplied by 1.4. This is approximately the relative training-induced increase in CS activity encountered in our study (see RESULTS, Muscle mitochondrial proteins). 3) In mode C the activity of OXPHOS is elevated by 5% in relation to mode A. In other words, the rate constants of all OXPHOS complexes and NADH supply are multiplied by 1.05. This is the relative training-induced increase (although not statistically significant) in COX activity encountered in our study (see RESULTS, Muscle mitochondrial proteins). 4) In mode D the rate constants of all OXPHOS complexes are multiplied by 1.05, as in mode C, and additionally the value of the ESA coefficient \( x \) is elevated from 3.5 to 4.1 in relation to mode A, to generate the expected acceleration of the muscle and pulmonary \( V\dot{O}_2 \) on-kinetics. This corresponds to an increase in the intensity of ESA. If we assume that there is in fact no training-induced increase in COX activity (the 5% increase is not statistically significant), a slightly greater increase in \( x \), and therefore in ESA, would be needed to decrease \( t_{0.63\text{on}} \) by about 19%.

Table 2. Values of selected cardiorespiratory variables and plasma lactate concentration obtained at exhaustion during the incremental exercise test performed before and after endurance training

<table>
<thead>
<tr>
<th>Variables</th>
<th>Before Training</th>
<th>After Training</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO(_2)peak, ml/min</td>
<td>3,187 ± 479</td>
<td>3,294 ± 323</td>
<td>0.21</td>
</tr>
<tr>
<td>VE, l/min</td>
<td>93 ± 17</td>
<td>100 ± 19</td>
<td>0.18</td>
</tr>
<tr>
<td>VCO(_2), ml/min</td>
<td>3,585 ± 521</td>
<td>3,855 ± 481</td>
<td>0.04</td>
</tr>
<tr>
<td>RER</td>
<td>1.13 ± 0.03</td>
<td>1.17 ± 0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>188 ± 8</td>
<td>188 ± 9</td>
<td>0.89</td>
</tr>
<tr>
<td>[La(^-)](_p), mmol/l (*)</td>
<td>8.1 ± 1.9</td>
<td>8.2 ± 1.8</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Data means ± SD. VE, minute ventilation; VCO\(_2\), minute CO\(_2\) production; RER, respiratory exchange ratio; HR, heart rate; [La\(^-\)]\(_p\), plasma lactate concentration. *End-exercise plasma lactate concentrations data are shown for 10 subjects. (P value was calculated using nonparametric Wilcoxon signed rank test).
Table 3. Pulmonary oxygen uptake on-kinetics during moderate-intensity cycling exercise before and after 20 wk of training

<table>
<thead>
<tr>
<th>Variables</th>
<th>Before Training</th>
<th>After Training</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline ( V \dot{O}_2 ), ml/min</td>
<td>799 ± 90</td>
<td>764 ± 64</td>
<td>0.02</td>
</tr>
<tr>
<td>( \tau_p ), s</td>
<td>28.3 ± 5.2</td>
<td>23.0 ± 4.0</td>
<td>0.005</td>
</tr>
<tr>
<td>( A_1 ), ml/min</td>
<td>656 ± 33</td>
<td>642 ± 44</td>
<td>0.31</td>
</tr>
<tr>
<td>Gain, ml·min(^{-1}·W(^{-1})</td>
<td>10.76 ± 0.53</td>
<td>10.52 ± 0.72</td>
<td>0.31</td>
</tr>
<tr>
<td>( EE V \dot{O}_2 )</td>
<td>1,456 ± 76</td>
<td>1,405 ± 74</td>
<td>0.001</td>
</tr>
<tr>
<td>% peak ( O_2 ) uptake</td>
<td>46.4 ± 6.0</td>
<td>42.9 ± 3.0</td>
<td>0.02</td>
</tr>
<tr>
<td>Power output, W</td>
<td>81 ± 0</td>
<td>81 ± 0</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Data means ± SD. (\( P \) value was calculated using nonparametric Wilcoxon signed rank test). \( A_1 \), asymptotic amplitude for the exponential term; \( \tau_p \), time constant of the primary \( V \dot{O}_2 \) on-kinetics; gain, \( \Delta V \dot{O}_2 \) in the steady state/\( \Delta \) power output; EE \( V \dot{O}_2 \), end-exercise oxygen uptake.

Statistics

The results presented in tables and figures are means ± SD. The significance of the difference between variables measured before and after the training was tested with the matched-pairs Wilcoxon signed-ranks test. Exact (i.e., nonasymptotic) two-sided \( P \) values were computed as described in Zoladz et al. (69). The nonparametric test with the exact \( P \) values was used because of the relatively small sample size (11 subjects), on which the comparisons were based. With 11 data points, it is not possible to reliably verify the assumption of normality, needed for an application of standard parametric tests, like the Student’s \( t \) test. For the nonparametric Wilcoxon signed-ranks test, one only needs to assume the symmetry of the distribution with respect to its median. Correlation analysis between two variables due to the small sample size was tested using nonparametric Spearman’s correlation analysis. The statistics were analyzed using the statistical package StatXact 9.0 (Cytel software, Cambridge, MA) and STATISTICA 9.0 (StatSoft, Tulsa, OK).

RESULTS

Cardiorespiratory Variables and Plasma Lactate Concentration

Training resulted in a nonsignificant (by about 3%) increase in absolute \( V \dot{O}_2\)peak (\( P = 0.21 \)) and in nonsignificant changes in minute ventilation (\( V \dot{E} \)), heart rate (HR), and [\( La^{-} \)]jpt determined at the end of the maximal incremental test (see Table 2). No effect of training on body mass was observed (75.48 ± 14.27 kg before training and 76.09 ± 12.94 kg (\( P = 0.39 \)) after training. Accordingly, no significant effect (\( P = 0.39 \)) of training on relative (per unit of body mass) peak \( V \dot{O}_2 \) values was found (42.8 ± 5.6 ml/kg before training and 43.9 ± 4.5 ml/kg after training). \( V \dot{O}_2 \) and respiratory exchange ratio (\( RER \)) reached at the maximal power output after training were significantly higher than before training (see Table 2). Power output at LT before training was 123 ± 30 W and after training it increased to 141 ± 28 W (\( P = 0.03 \)).

Oxygen Uptake Kinetics

Baseline \( V \dot{O}_2 \) after training decreased by −4\% (\( P = 0.02 \)) (see Table 3). The \( \tau_p \) of the phase II of the pulmonary \( V \dot{O}_2 \) on-kinetics decreased after training by −19\% (\( P = 0.03 \)) (Table 3). The end-exercise \( V \dot{O}_2 \) corresponded to 46.4\% and 42.9\% of \( V \dot{O}_2\)peak respectively, before and after training (\( P = 0.02 \)) (Table 3). No significant effect of training on the phase II amplitude and phase II gain was found (Table 3). The end-exercise \( V \dot{O}_2 \) after training was by 3.5\% lower (\( P = 0.001 \)) than before training (Table 3).

A significant decrease (\( P = 0.0005 \)) in the baseline of the pulmonary \( V \dot{O}_2 \) off-kinetics was observed after training (Table 4). Phase II \( \tau_p \) of the pulmonary \( V \dot{O}_2 \) off-kinetics decreased after training by −11\% (\( P = 0.02 \)) (Table 4). No significant effect of training on the phase II amplitude was found (Table 4).

Before training, the \( \tau_p \) of the pulmonary \( V \dot{O}_2 \) on-kinetics (28.3 ± 5.2 s) tended to be lower than the \( \tau \) of the pulmonary \( V \dot{O}_2 \) off-kinetics (33.7 ± 7.2 s), but this difference was statistically not significant (\( P = 0.07 \)); in other words, no “asymmetry” was observed. After training, however, \( \tau_p \) of the pulmonary \( V \dot{O}_2 \) on-kinetics (23.0 ± 4.0 s) was significantly (\( P = 0.001 \)) lower than the \( \tau_p \) of the pulmonary \( V \dot{O}_2 \) off-kinetics (30.0 ± 6.6 s), i.e., asymmetry was observed.

mtDNA Content and Mitochondrial Protein Content and Activity

Muscle mitochondrial DNA copy number. Training resulted in a significant (\( P = 0.03 \)) (−53\%) increase in mtDNA copy number in relation to nuclear DNA (mtDNA/nDNA) (from 3,039 ± 734 to 4,639 ± 1,308, respectively, before and after training) (Fig. 2D).

Muscle mitochondrial proteins. Maximal CS activity after training increased by 38\%, i.e., from 7.24 ± 1.62 nmol-min\(^{-1}·mg\) protein\(^{-1}\) to 9.98 ± 1.84 nmol TNB-min\(^{-1}·mg\) protein\(^{-1}\) (\( P = 0.002 \)) (Fig. 2A). Maximal COX activity tended to be higher (by −5\%, \( P = 0.08 \)) after training, i.e., from 4,729 ± 586 nmol O\( _2\)-min\(^{-1}·mg\) protein\(^{-1}\) to 4,960 ± 577 nmol O\( _2\)-min\(^{-1}·mg\) protein\(^{-1}\) (Fig. 2B). Twenty weeks of moderate-intensity endurance training resulted in a significant increase (by −38\%) of CS content (\( P = 0.004 \)) (Fig. 2C).

Myosin Heavy Chain Composition in the Vastus Lateralis.

The percentages of slow MyHC (MyHC-1) and fast MyHC (MyHC-2) in the vastus lateralis before endurance training were 40.1 ± 14.4\% and 59.1 ± 14.4\%, respectively, for MyHC-1 and MyHC-2. After 20-wk endurance training no significant changes (\( P = 0.13 \)) (45.4 ± 15.6\% vs. 54.6 ± 15.6\%, respectively, for MyHC-1 and MyHC-2) were observed. No significant correlations between the training-induced changes in \( \tau_p \) of the pulmonary \( V \dot{O}_2 \) on-kinetics (\( \Delta \tau \)) and the training-induced changes in MyHC slow (MyHC slow) (\( r = 0.22, P = 0.53 \)) or \( \Delta \)MyHC fast (\( r = −0.22, P = 0.53 \)) were found.

Table 4. Pulmonary oxygen uptake off-kinetics during moderate-intensity cycling exercise before and after 20 wk of training

<table>
<thead>
<tr>
<th>Variables</th>
<th>Before Training</th>
<th>After Training</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline ( O_2 ) uptake, ml/min</td>
<td>1,464 ± 66</td>
<td>1,382 ± 92</td>
<td>0.0005</td>
</tr>
<tr>
<td>( \tau_p ), s</td>
<td>33.7 ± 7.2</td>
<td>30.0 ± 6.6</td>
<td>0.02</td>
</tr>
<tr>
<td>( A_1 ), ml/min</td>
<td>646 ± 110</td>
<td>637 ± 62</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Data are means ± SD. (\( P \) value was calculated using nonparametric Wilcoxon signed rank test). \( \tau_p \), time constant of the primary \( V \dot{O}_2 \) off-kinetics.
Correlations Between $\Delta \tau_p$ and Changes in Markers of Mitochondrial Biogenesis

We have also tried to correlate $\Delta \tau_p$ of the pulmonary $\dot{V}_O_2$ on-kinetics with the training-induced changes in the markers of muscle mitochondrial biogenesis. No significant correlations were found between $\Delta \tau_p$ of the pulmonary $\dot{V}_O_2$ on-kinetics and $\dot{V}_O_2$ ($r = 0.09$, $P = 0.80$), $r = 0.07$, $P = 0.85$), or $\Delta$ CS activity ($r = 0.50$, $P = 0.14$). No significant correlation ($r = 0.07$, $P = 0.85$) was also found between $\Delta \tau_p$ of the pulmonary $\dot{V}_O_2$ on-kinetics and $\dot{V}_O_2$ COX protein amount.

Computer Simulations

The employed computer simulations show that, for the assumed ESA coefficient $x$ value (0.35), muscle $t_{0.63on}$ would be about 30 s, which is in accordance with our experimental data for pulmonary $\dot{V}_O_2$ on-kinetics (see Table 5 and Fig. 3). Within the model OXPHOS activity is defined as the relative rate constants of OXPHOS complexes. An increase in OXPHOS activity by 40%, which approximately corresponds to the training-induced increase in CS activity in our study, would cause a decrease in $t_{0.63on}$ by 17%, as shown in Table 5 and Fig. 3A (mode A–mode B transition). This value is very similar to the decrease in $\tau_p$ we experimentally observed (~19%). If OXPHOS activity is increased by only 5%, which corresponds to the maximal value of the training-induced increase in COX activity in our study, $t_{0.63on}$ would decrease by only 1.9% (see Table 5 and Fig. 3B) (mode A–mode C transition). This value appears substantially lower than the decrease in $\tau_p$ we experimentally observed. On the other hand, if ESA is intensified ($x$ coefficient increases from 0.35 to 0.41) in the presence of an increase of OXPHOS activity by 5%, $t_{0.63on}$ would drop by 18%, as can be seen in Table 5 and Fig. 3B (mode A–mode D transition). If we assume no increase in COX activity (we observed a statistically nonsignificant tendency of COX activity increase), then a slightly larger increase in $x$ coefficient would be needed to decrease $t_{0.63on}$ by about 19%. As with

<table>
<thead>
<tr>
<th>Mode</th>
<th>$t_{0.63on}$, s</th>
<th>$t_{0.63off}$, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mode A (untrained)</td>
<td>31.3</td>
<td>23.0</td>
</tr>
<tr>
<td>Mode B (40% up OXPHOS activity)</td>
<td>26.0 (17%)</td>
<td>19.3 (16%)</td>
</tr>
<tr>
<td>Mode C (5% up OXPHOS activity)</td>
<td>30.7 (19%)</td>
<td>22.5 (22%)</td>
</tr>
<tr>
<td>Mode D (5% up OXPHOS activity, ↑ ESA)</td>
<td>25.9 (18%)</td>
<td>21.0 (19%)</td>
</tr>
</tbody>
</table>

OXPHOS, oxidative phosphorylation; ESA, each-step activation; ↑, increase in relation to mode A; ↓, decrease in relation to mode A.
maximal COX activity was only slightly (by 5%) increased ($P = 0.08$). By using our computer model of the bioenergetic system in skeletal muscle developed previously (35, 36), we quantitatively estimated the effects of the training-induced changes in OXPHOS activity and ESA (each step activation) intensity (65) on the observed acceleration of the pulmonary VO$_2$ on- and off-kinetics. The main novel finding of the present study is that the main or only mechanism responsible for the acceleration of pulmonary (and muscle) VO$_2$ on-kinetics following prolonged endurance training of moderate-intensity as applied in the present study is an increase in ESA intensity.

**Maximal CS and COX Activities and Pulmonary VO$_2$ On-Kinetics**

In the present study the training-induced increase in maximal CS activity was significant (~38%), whereas the effect on maximal COX activity (~5%) was very small, if any, at all (the increase was not statistically significant). However, it should be remembered that we have applied an endurance training program composed of four sessions per week of a rather moderate workload, since ~86% of its total duration was performed below LT (~50% of VO$_{2\max}$) and only ~14% of its total duration was performed in the heavy-intensity domain (~75% of VO$_{2\max}$). Wibom et al. (60) adopted an endurance training program lasting 6 wk composed of high-intensity training sessions corresponding to 70% of VO$_{2\max}$ performed 6 times per week, and observed an increase of maximal COX activity by 78% and an increase of maximal CS activity by 40%. This illustrates that the training workload has a strong effect on the magnitude of changes in muscle mitochondrial enzymes activities and that the training-induced changes in maximal CS and COX activities can vary substantially depending on the training protocol [for overview of this point see also Holloszy (23) and Holloszy and Coyle (24)]. Therefore, our results cannot be considered as representative for all kinds of training. Moreover, it has been recently reported by Perry et al. (51) that the training-induced increase in muscle CS mRNA preceded the increase in COX-IV mRNA. This suggests that muscle CS is more sensitive to physical training than COX. This could be also a potential cause of the significant differences in the training-induced changes of CS and COX activities observed in the present study.

Both the increase in maximal in vitro CS and COX activities are regarded as markers of mitochondria biogenesis (23, 24, 28). A relevant problem, in the context of the present study, is which of them can be considered a better measure of OXPHOS activity in vivo. Since the activity increases following training in the present study were very different, it cannot be that both enzymes are equally contributing to the observed functional changes. The question is relevant in terms of the mechanisms potentially responsible for the acceleration of pulmonary VO$_2$ on-kinetics induced by training.

If one assumes that the shortening of $\tau_p$ is exclusively due to an increase of the OXPHOS activity caused by/proportional to an increase in CS activity, it means that the acceleration of the muscle and pulmonary VO$_2$ on-kinetics should be due to an about 1.4-fold (by ~40%) increase in OXPHOS activity. We used our computer model to test this possibility. Within the model the effect of training in this case would be equivalent to the transition from mode $A$ to mode $B$. One can see from Fig.

**DISCUSSION**

In the present study we have found that in healthy and previously untrained young men a prolonged (20 wk) endurance training program comprising both moderate- and high-intensity exercise resulted in a significant shortening (by ~19%) of the $\tau_p$ of the pulmonary VO$_2$ on-kinetics during moderate-intensity cycling. Moreover, the adopted training resulted also in a significant shortening (by ~11%) of the $\tau_p$ of the pulmonary VO$_2$ off-kinetics, thereby determining an asymmetry between the pulmonary VO$_2$ on- and off-kinetics. These effects were accompanied by significant increases of some markers of muscle mitochondrial biogenesis in the vastus lateralis muscle, such as the mtDNA copy number (mtDNA/nDNA) (increased by ~53%), maximal CS activity (increased by 38%), and CS protein content (increased by 38%), whereas
3A and Table 5 that the simulated acceleration of muscle $V_o_2$ on-kinetics (17%) would be similar to the acceleration of pulmonary $V_o_2$ on-kinetics experimentally observed in our study (19%). This would lead to the conclusion that an increase in the OXPHOS activity, related to CS activity, is indeed the main or only mechanism responsible for the observed acceleration of the pulmonary $V_o_2$ on-kinetics. On the other hand, if COX activity is a better measure of OXPHOS activity, then the increase of OXPHOS activity by maximum 5% (mode A-mode C transition) would lead to a decrease of $\tau_p$ for muscle and pulmonary $V_o_2$ on-transient by maximum only 1.9%. This value is much smaller than the observed 19% decrease. At a first look this observation seems to exclude the possibility that OXPHOS activity is proportional to COX activity, would support the concepts that CS activity is a better measure of OXPHOS activity, and that the latter determines muscle and pulmonary $V_o_2$ on-kinetics.

However, we can envisage a serious reason leading us to doubt this conclusion. Namely, CS would have essentially no control over $V_o_2$ (its flux control coefficient, FCC, see Eq. A1 in APPENDIX, is very close to zero). This is because OXPHOS (including COX) in skeletal muscle mitochondria keeps essentially all the control over $V_o_2$; the sum of FCCs for OXPHOS complexes is very close to 1 (55). The summation property (see APPENDIX, Eq. A2) says that the values of FCCs of all enzymes sum up to unity, and that this value cannot be exceeded. In the mentioned experiment (55) isolated skeletal muscle mitochondria were incubated with pyruvate-malate, and therefore most tricarboxylic acid (TCA) cycle enzymes, including CS, were involved in respiration. However, OXPHOS complexes left essentially no space for the control over $V_o_2$ by other enzymes/ parts of the system. For this reason it is very likely that the TCA cycle in general has a negligible control over $V_o_2$, and therefore even a strong activation of one or several of its enzymes would not cause any noticeable stimulation of $V_o_2$. In other words, the training-induced increase in CS activity by 38% would have essentially no effect in the control of OXPHOS activity and muscle $V_o_2$ on-kinetics.

If we assume that, as reported recently by Larsen et al. (42), COX activity is the best measure of OXPHOS activity (in other words, the increase of the activity of other OXPHOS complexes as a result of training is of comparable extent as that of COX activity), the problem remains which mechanism causes the acceleration of $V_o_2$ on-kinetics by 19%, and not by maximum 1.9% resulting from the increase in OXPHOS activity by maximum 5%. A likely candidate would be the ESA mechanism (32, 33, 34) proposed by our group as the only mechanism responsible for the shortening $\tau_p$ following short-term moderate-intensity exercise training (65). The potential efficiency of this mechanism is demonstrated in Fig. 3B and Table 5, in which mode D corresponds to a state where OXPHOS activity is increased by 5%, and the intensity of each-step activation is elevated (ESA coefficient $x$ is increased from 0.35 to 0.41 in relation to mode A). In mode D $l_o_{6.63s}$ is shorter by 18% than in mode A, which is very similar to the training-induced decrease of $\tau_p$ (19%) observed in our study. Thus a joint mechanism involving both an increase in OXPHOS activity and an increase in ESA intensity would be accountable for the decreased $\tau_p$ observed in the present study. If we assume that there is actually no increase in COX activity (the observed difference was statistically not significant), a slightly greater increase in ESA intensity would be needed to decrease to 0.63$s$ by about 19%.

It must be emphasized that COX keeps only about 20% of the metabolic control over $V_o_2$ in state 3 in skeletal muscle mitochondria (its flux control coefficient equals 0.2), whereas the rest of the control is exerted by the remaining OXPHOS complexes (55). Therefore, to draw our conclusions, we must assume (which seems to be a reasonable assumption) that the activity of these complexes would also increase by about 0–5% as a result of training. Of course, the control exerted by COX in vivo can be different from the control in isolated mitochondria in state 3 (we mean relative control among OXPHOS complexes, because in intact muscle most of the control is at ATP usage). However, this is the best estimation of which we know.

It must be emphasized that ESA is active only during muscle work but not at rest or in isolated mitochondria. Therefore, the increase in COX activity caused by ESA during muscle work is something completely different from the increase in COX amount/activity caused by an increase in mitochondria biogenesis. Both effects add up during muscle work.

Our computer simulations allow us to estimate the relative contribution of the two proposed mechanisms, the increase in OXPHOS activity and the increase in ESA intensity, to the overall shortening of $\tau_p$. Namely, the contribution of the increase in OXPHOS (including COX) activity would be 0 s/19 s = 0 (0%) to 1.9 s/19 s = 0.1 (10%), whereas the contribution of the increase in ESA intensity would be 0.9 (90%) to 1.0 (100%). We regard these values only as a rough approximation, because we did not measure the increase in the activity of other (than COX) OXPHOS complexes, and assumed that such increases, if present, would be similar to the observed increase in COX activity (see above).

While all OXPHOS complexes are directly activated by Ca$^{2+}$ (16), CS does not seem to be directly activated by Ca$^{2+}$. Probably this is because it has a very low FCC, as discussed above, and therefore, its rate can be very effectively increased by changes in its substrates and products concentrations (FCCs are inversely proportional to enzyme sensitivity to metabolite concentrations). It is also possible that the training-induced increase in its activity constitutes an additional “capacity buffer.”

Percentage of Muscle MyHC-1 and Pulmonary $V_o_2$ On-Kinet
cs

It has been reported previously, that the percentage of type I muscle fibers in the vastus lateralis is negatively correlated with the $\tau_p$ of the pulmonary $V_o_2$ on-kinetics during heavy exercise (54). Therefore, in the present study, we have also evaluated the effect of the applied endurance training on the percentages of MyHC-1 and MyHC-2 in the vastus lateralis muscle. We have found, however, that the training-induced acceleration of the pulmonary $V_o_2$ on-kinetics was accompanied by no significant changes in percentage of MyHC-1. This is in accordance with previous studies (58, 65). Moreover, in the present study we have found no significant correlations between the training-induced changes in the $\tau_p$ of the pulmonary $V_o_2$ on-kinetics with the training-induced changes in MyHC slow and fast isoforms. This illustrates that the training-induced acceleration of the $\tau_p$ of the pulmonary $V_o_2$ on-kinetics is something completely different from the increase in COX amount/activity caused by an increase in mitochondria biogenesis. Both effects add up during muscle work.

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It has been reported previously, that the percentage of type I muscle fibers in the vastus lateralis is negatively correlated with the $\tau_p$ of the pulmonary $V_o_2$ on-kinetics during heavy exercise (54). Therefore, in the present study, we have also evaluated the effect of the applied endurance training on the percentages of MyHC-1 and MyHC-2 in the vastus lateralis muscle. We have found, however, that the training-induced acceleration of the pulmonary $V_o_2$ on-kinetics was accompanied by no significant changes in percentage of MyHC-1. This is in accordance with previous studies (58, 65). Moreover, in the present study we have found no significant correlations between the training-induced changes in the $\tau_p$ of the pulmonary $V_o_2$ on-kinetics with the training-induced changes in MyHC slow and fast isoforms. This illustrates that the training-induced acceleration of the $\tau_p$ of the pulmonary $V_o_2$ on-kinetics is something completely different from the increase in COX amount/activity caused by an increase in mitochondria biogenesis. Both effects add up during muscle work.

Our computer simulations allow us to estimate the relative contribution of the two proposed mechanisms, the increase in OXPHOS activity and the increase in ESA intensity, to the overall shortening of $\tau_p$. Namely, the contribution of the increase in OXPHOS (including COX) activity would be 0 s/19 s = 0 (0%) to 1.9 s/19 s = 0.1 (10%), whereas the contribution of the increase in ESA intensity would be 0.9 (90%) to 1.0 (100%). We regard these values only as a rough approximation, because we did not measure the increase in the activity of other (than COX) OXPHOS complexes, and assumed that such increases, if present, would be similar to the observed increase in COX activity (see above).

While all OXPHOS complexes are directly activated by Ca$^{2+}$ (16), CS does not seem to be directly activated by Ca$^{2+}$. Probably this is because it has a very low FCC, as discussed above, and therefore, its rate can be very effectively increased by changes in its substrates and products concentrations (FCCs are inversely proportional to enzyme sensitivity to metabolite concentrations). It is also possible that the training-induced increase in its activity constitutes an additional “capacity buffer.”

Percentage of Muscle MyHC-1 and Pulmonary $V_o_2$ On-Kinet
cs

It has been reported previously, that the percentage of type I muscle fibers in the vastus lateralis is negatively correlated with the $\tau_p$ of the pulmonary $V_o_2$ on-kinetics during heavy exercise (54). Therefore, in the present study, we have also evaluated the effect of the applied endurance training on the percentages of MyHC-1 and MyHC-2 in the vastus lateralis muscle. We have found, however, that the training-induced acceleration of the pulmonary $V_o_2$ on-kinetics was accompanied by no significant changes in percentage of MyHC-1. This is in accordance with previous studies (58, 65). Moreover, in the present study we have found no significant correlations between the training-induced changes in the $\tau_p$ of the pulmonary $V_o_2$ on-kinetics with the training-induced changes in MyHC slow and fast isoforms. This illustrates that the training-induced acceleration of the $\tau_p$ of the pulmonary $V_o_2$ on-kinetics is something completely different from the increase in COX amount/activity caused by an increase in mitochondria biogenesis. Both effects add up during muscle work.
kinetics cannot be explained by the training-induced changes in MyHC composition in the vastus lateralis muscle.

**Mitochondrial Biogenesis and Increase in OXPHOS Activity**

We do not discuss the mitochondrial DNA copy number in this context, because this is not a "kinetic" parameter, and therefore it does not have any direct impact on OXPHOS. While COX and CS activities have a direct influence on the kinetic behavior of the system, the relation between mtDNA copy number, mRNA amount, enzyme amount, enzyme activity, and finally kinetics of the system is very indirect and not unique (e.g., one can imagine a significant increase in mtDNA without an increase in enzyme amount/activity; this would have no impact on metabolism). We presented the effect of training on mtDNA copy number (and CS activity) to show that not all markers of "mitochondria biogenesis" are good indicators of OXPHOS activity. These generally accepted markers of "mitochondria biogenesis" can increase in the presence of no changes in the OXPHOS activity; therefore, the assessment of training-induced increase in OXPHOS capacity/activity based on measurements of mtDNA copy number and CS activity can be misleading (for overview of this point see also Ref. 42).

Throughout this article we use the term "increase in OXPHOS activity," and not "increase in mitochondrial biogenesis." We do this on purpose because in our opinion the concept of increase in mitochondrial biogenesis is very vague. The increase in mitochondrial biogenesis seems indeed to have many aspects, which would not occur with the same intensity, as shown by the big difference between the increase in mtDNA copy number, CS activity, and in COX activity seen in our study. Of course, the aspect we are most interested in is that one is related to OXPHOS activity and muscle/pulmonary V˙O₂ on-kinetics, but there are others. The so-called increase in mitochondrial biogenesis has many markers comprising mtDNA, CS activity, COX activity, HAD activity, mitochondrial volume, mitochondrial membranes (see e.g., Refs. 15, 23, 24, 26, 28, 46, 47, 59). The changes of these variables in response to training show different rates and magnitudes (see e.g., Refs. 47, 51, 60). For example, it was recently reported by Perry et al. (51) that heavy interval training rapidly induced CS and β-HAD mRNA increase (after first training session), then CS and β-HAD activities mitochondrial DNA increase, next COX-IV mRNA increase, and finally after the fifth training session, COX-IV protein increase. In our opinion there is not a single measure of "increase in mitochondrial biogenesis," which represents a very heterogeneous process.

**Isolated Mitochondria Versus Intact Muscle System**

Of course, there exist several important differences between the isolated mitochondria and intact skeletal muscle systems. Perhaps two of them are most relevant for the present study. First, in vivo, most of the control over V˙O₂ is exerted by ATP usage, and therefore the distribution of the control in state 3 in isolated mitochondria can be extrapolated only to the relative distribution of control within the ATP supply block (OXPHOS, TCA cycle, glycolysis) in intact skeletal muscle (compare Ref. 34). For this reason, an increase in any enzyme activity within the ATP supply block, for instance, in CS or COX activity would have a very small impact on V˙O₂ (additionally, the control of CS is close to zero even in state 3 in isolated mitochondria; see above) (this does not contradict the fact that an increase in OXPHOS activity accelerates the muscle V˙O₂ on-kinetics and increases intermediate metabolite homeostasis during work transitions). Second, in isolated mitochondria (at least in the absence of Ca²⁺) there is of course no ESA; the only regulatory mechanism is the negative feedback through ADP (state 4–state 3 transition). Additionally, the presence of the CK system (CK + PCr + Cr) significantly slows down the muscle V˙O₂ on-kinetics; in the presence of CK τₚ is inversely proportional to the size of the total creatine (PCr + Cr) pool (38).

**Significance of the Training-Induced Acceleration of the Muscle V˙O₂ On-Kinetics**

The acceleration of pulmonary V˙O₂ on-kinetics observed in the present study is functionally and physiologically relevant. In previous theoretical studies (37, 38) we have shown, using a computer model of the bioenergetic system in skeletal muscle (36), that the training-induced acceleration of the muscle V˙O₂ on-kinetics could be considered an indirect marker of an enhancement of muscle metabolic stability during exercise at a given power output. We have demonstrated that at a given ATP demand and under conditions where the CK reaction works near the thermodynamic equilibrium, the half-transition time t₀.₅ (and thus also τₚ) of the muscle V˙O₂ on-kinetics is determined by the amount of PCr that has to be transformed into creatine (Cr) during the rest-to-work transition (38) (see also Fig. 3B therein). This is in agreement with the results of experimental studies showing a significant positive correlation between the magnitude of decrease in muscle PCr concentration during exercise and the rate of the pulmonary V˙O₂ on-kinetics in humans (2, 52). We have postulated that the training-induced shortening of τₚ of the muscle V˙O₂ on-kinetics is a consequence of muscle-adaptive responses resulting in attenuation of the magnitude of a decrease in muscle PCr level (∆PCr in mM) during rest-to-work transition (38). This is accompanied by a lowering of the exercise-induced disturbances in other muscle metabolites, e.g., ADP, Pi, H⁺, IMP, AMP, and consequently ∆G ATP (19, 37, 38, 67). An improvement of muscle metabolic stability during exercise should be beneficial for enhancement of exercise tolerance and resistance to fatigue (for review see, Refs. 11, 61, 64, 66, 67).

We postulated recently (65) that in the early stage (days or weeks) of moderate-intensity training the acceleration of the pulmonary (and muscle) V˙O₂ on-kinetics is almost exclusively reached by an enhancement of ESA in skeletal muscles. In the case of prolonged (months) (present study) endurance training of mainly moderate intensity, it seems that an enhancement of OXPHOS activity can also play a small role in the acceleration of the kinetics. The question remains in terms of the relative contribution of the enhanced ESA and increase of OXPHOS activity in providing the exceptionally high muscle metabolic stability (21, 22, 45) and extremely fast pulmonary V˙O₂ on-kinetics (τₚ < 10 s) occurring in extremely well-trained elite endurance athletes following several years of endurance training (for review see Ref. 30). One can speculate that the contribution of the increase in the OXPHOS activity to the decrease in τₚ will increase with the training length and its intensity. Wibom et al. (60) showed that as little as 6 wk of...
high-intensity cycling can increase COX activity by about 80%, suggesting, when compared with our study, that appropriate training intensity can be more important than training duration for the increase in OXPHOS activity (for overview of this point see also Ref. 23). In endurance athletes the increase of OXPHOS activity after several years of training could be even greater (see also Ref. 24).

The training-induced acceleration of the pulmonary VO₂ on-kinetics in the present study was accompanied by a significant increase in the power output at the LT. The training-induced increase in ESA activity may result both in an acceleration of the muscle and pulmonary VO₂ on-kinetics and in a shifting the LT into higher power outputs, by increasing ATP supply from oxidative phosphorylation and decreasing ATP supply from anaerobic glycolysis, thereby enhancing muscle metabolic stability at a given power output.

**Effect of Endurance Training on Muscle and Pulmonary VO₂ Off-Kinetics**

In the present study we have also evaluated, using our computer model, muscle VO₂ off-kinetics and the effect of training on this kinetics. The muscle VO₂ on- and off-kinetics are related to each other, among others, through the intensity of ESA. In our opinion the problem of both on- and off-kinetics, and the (as)ymmetry between them is interesting by itself, especially that all three properties of the system are affected by muscle training. For a realistic value of the characteristic decay time of ESA after termination of exercise \( \tau(\text{OFF}) = 100 \) s (compare Eq. 4) (it gives a realistic value of \( t_{0.63} \) for PCr off-kinetics, equal to 25–37 s depending on work intensity, see Ref. 39), we estimated in mode A (untrained muscle), \( t_{0.63} \) for the muscle VO₂ off-transient, which would be about 27% lower than the \( t_{0.63} \) for the on-transient (23.0 s vs. 31.3 s, see Table 4). This value is similar to the value of 31% for the difference between muscle VO₂ on- and off-kinetics obtained experimentally by Krstrup and coworkers (40) in humans in thigh muscles during leg-kicking exercise.

Both an increase of muscle mitochondrial OXPHOS activity and increase in ESA intensity would result in a shortening of \( t_{0.63} \) of the muscle VO₂ off-kinetics (see Table 5). We have recently demonstrated that the \( t_{0.63} \) of the muscle VO₂ off-kinetics is strongly dependent on \( \tau(\text{OFF}) \) (39). Namely, an increase of \( \tau(\text{OFF}) \) would slow down the initial phase of the muscle VO₂ off-kinetics and would accelerate muscle PCr off-kinetics (39). This suggests that training-induced changes in muscle OXPHOS activity, in ESA intensity, and possibly in \( \tau(\text{OFF}) \) would codetermine changes in muscle VO₂ off-kinetics in humans.

To our best knowledge no experimental data has been published so far on the effects of endurance training on muscle VO₂ off-kinetics in humans. It has been reported that a few weeks of endurance training results in a significant shortening of the \( \tau_p \) of the pulmonary VO₂ off-kinetics in humans (13). Also in the present study we have demonstrated that the applied endurance training resulted in a significant shortening (by about 11%) of the \( \tau_p \) of the pulmonary VO₂ off-kinetics. However, it must be remembered that the pulmonary VO₂ off-kinetics is about twice slower than the muscle VO₂ off-kinetics at moderate exercise (40) and therefore other factors than the muscle off-kinetics determine the pulmonary VO₂ off-kinetics (see the next paragraph).

Regarding the relationship between pulmonary VO₂ on- and off-kinetics, the so-far published data are ambiguous. Some authors reported that \( \tau_p \) of the pulmonary VO₂ on-kinetics during moderate-intensity exercise is similar to the \( \tau_p \) of the pulmonary VO₂ off-kinetics (symmetry) (see e.g., Refs. 43, 49, 50). On the other hand, other researchers reported an asymmetry of the two kinetics in moderate-intensity exercise (the pulmonary on-kinetics faster than pulmonary off-kinetics) (see e.g., Refs. 7, 27, 57). We suggested recently (39) that the pulmonary VO₂ off-kinetics is determined not only by the muscle VO₂ off-kinetics, but also by other factors (which would slow down the pulmonary VO₂ off-kinetics), such as a slowly decreasing oxygen consumption by heart and respiratory muscles (more slowly than oxygen consumption by locomotory muscles) or gradually slowing-down oxygen transport by blood during recovery after exercise.

A comparison of computer simulations with experimental data, as well as the above discussion, suggest that the effect of training on pulmonary VO₂ on-kinetics can be fully explained by the effect on muscle VO₂ on-kinetics, and that the effect on pulmonary VO₂ off-kinetics can be only partly explained by the effect on muscle VO₂ off-kinetics.

In the present study we observed that the training-induced decrease in pulmonary VO₂ on-kinetics is greater than the decrease in pulmonary VO₂ off-kinetics. For this reason an on/off symmetry of pulmonary VO₂ kinetics takes place before training and on/off asymmetry takes place after training. Our results suggest that the training status of the subjects should be considered when studying the relationship between pulmonary VO₂ on- and off-kinetics in humans. However, for the reasons discussed above, the pulmonary on/off symmetry/asymmetry says us little about the muscle on/off symmetry/asymmetry.

Generally, Krstrup et al. (40) measured a “normal” pulmonary on/off asymmetry (VO₂ on-kinetics faster than VO₂ off-kinetics) and “inverse” muscle on/off asymmetry (VO₂ on-kinetics slower than VO₂ off-kinetics). We, similarly, measured either on/off symmetry (with tendency to “normal” on/off asymmetry) (untrained subjects) or “normal” on/off asymmetry (trained subjects) for pulmonary VO₂ kinetics and simulated “inverse” on/off asymmetry for muscle VO₂ kinetics.

**Limitations of Present Study**

The adopted prolonged endurance training (lasting 20 wk) was mainly composed by moderate-intensity cycling. This training was sufficient to significantly accelerate the VO₂ on-kinetics and to increase significantly mtDNA and CS amount/activity, but it was not effective in increasing maximal COX activity. We have argued, using metabolic control analysis, that COX activity is best correlated with OXPHOS activity. Therefore, we have postulated that the observed acceleration of the pulmonary VO₂ on-kinetics was almost exclusively due to training-induced enhancement of ESA (each-step activation). However, as discussed above, training at higher intensities (23, 24, 60) can increase maximal COX activity to much greater extent than the training applied in our study. Therefore, in the case of high-intensity training, the importance of the training-induced increase in OXPHOS activity in determining the...
acceleration of the muscle and pulmonary \( V_{O2} \) on-kinetics could be greater than in the present study.

In the present study the training-induced changes in muscle OXPHOS activity were assessed based on measurements of maximal COX activity, which, according to the recent study by Larsen et al. (42) is the most valid biomarker of muscle OXPHOS capacity. However, direct measurement of the training-induced changes in muscle OXPHOS activity (e.g., by measurement of maximum respiration rate in permabilized muscle fibers) would allow a more direct evaluation of the variable of interest.

Recent works point out that also \( O_2 \) delivery [see the review by Murias et al. (48)] and/or the intramuscular matching of \( O_2 \) delivery and \( O_2 \) utilization (31) may contribute as limiting/controlling factors of the muscle \( V_{O2} \) on-kinetics. Therefore, it cannot be excluded that prolonged training could accelerate the pulmonary \( V_{O2} \) on-kinetics, at least in part also by acting on \( O_2 \) delivery or on the intramuscular \( O_2 \) delivery-\( O_2 \) utilization matching.

The computer model of muscle bioenergetic system used in the present study is, as every model, only an approximation of the complex reality. It has been broadly validated by comparison with experimental data (see e.g., Refs. 33, 34), but it cannot take into account all relevant details. For instance the assumption that there is no anaerobic glycolysis during moderate exercise, especially at the onset of exercise, is not 100% true, as reported by e.g., Cerretelli et al. (8), and because of this, NADH supply and \( H^+ \) production that shifts CK equilibrium toward \( Cr \) production are underestimated in the model, and this could slightly affect our estimation of muscle \( V_{O2} \) on-kinetics. Nevertheless, the simulated muscle \( V_{O2} \) on-kinetics is very close to exponential, in accordance with experimental data, which testifies that the discussed effects are minor. As to interpretation of our results, we do not take into account the possible effect of microvascular \( O_2 \) delivery limitations on the \( V_{O2} \) on-kinetics, as discussed above.

Conclusions

Generally, in the present study we have found that an endurance training lasting 20 wk resulted in a significant acceleration of the pulmonary \( V_{O2} \) on- and off-kinetics during moderate exercise (by 19% and 11%, respectively). The applied training resulted also in an increase in some markers of mitochondrial biogenesis including mtDNA copy number (by 53%), maximal CS activity, and CS protein content (both by 38%). The maximal COX activity tended to increase (by about 5%), but this effect was not statistically significant. After assuming, on the basis of experimental studies carried out by applying MCA to isolated skeletal muscle mitochondria, that COX activity is a much better measure of OXPHOS activity than CS activity, we have estimated, by using a computer model of skeletal muscle bioenergetics, that in the present study the training-induced increase in OXPHOS activity accounts for about 0–10% of the decrease in \( \tau_p \) of muscle \( V_{O2} \) for on-transient, whereas the remaining 90–100% is caused by an increase in the intensity of ESA of OXPHOS complexes.

It is worth noticing that, as our present study shows, there is no simple correlation between the relative increase in mitochondria biogenesis markers, such as mtDNA, CS content/activity or COX activity, and \( \tau_p \). First, different markers change to very different extents, so they cannot all determine \( \tau_p \). A significant decrease in \( \tau_p \) can be even observed without any signs of increase in mitochondria biogenesis (65). Second, as our theoretical studies show, ESA can strongly decrease \( \tau_p \). The emphasis of the role of an increase in ESA intensity constitutes the most novel and important achievement of the present paper.

Perspectives and Significance

The present study emphasizes the potential importance of the ESA mechanism of the regulation of OXPHOS during work transitions that was proposed on the basis of theoretical studies (32, 33, 34) and recently received experimental support (16). It also demonstrates the usefulness of well-validated computer models in predicting the existence of phenomena that have not been previously discovered in experimental studies, as well as in the quantitative evaluation of experimental data. In the future it should be determined what is the contribution of different mechanisms (e.g., increase in ESA intensity, increase in OXPHOS activity, improvement of \( O_2 \) delivery) to the acceleration of muscle and pulmonary \( V_{O2} \) on-kinetics following training of different intensities and durations.

APPENDIX

Metabolic Control Analysis: Flux Control Coefficient

To estimate a potential influence of a given enzyme activity on the flux through the system, for instance of CS activity, on \( V_{O2} \), it is necessary to know the control exerted by this enzyme over the flux. The best means to quantify the extent to which a given enzyme (or process or metabolic block) controls the metabolite flux through a given metabolic pathway is MCA (for review see Ref. 9). The contribution of particular enzymes to the control over the metabolite flux is quantified by a parameter called flux control coefficient (FCC). It is defined as the ratio of the relative change in a given flux caused by a small relative change in the enzyme concentration/activity to this change itself. For enzyme \( E_i \) and flux \( J \) we have the following expression:

\[
C^i_F = \frac{\partial J/J}{\partial E_i/E_i} \quad (A1)
\]

where \( \partial J/J \) is a relative change in flux and \( \partial E_i/E_i \) is a small relative change in enzyme \( i \) concentration/activity. The values of flux control coefficient of all enzymes in a given (linear) pathway sum up to unity (the so-called summation property for FCCs).

\[
\sum_{i=1}^{n} C^i_F = 1 \quad (A2)
\]

Therefore, in complex systems composed of many enzymes, the values of flux control coefficients of most enzymes must be very small. MCA practically excludes the so-called “rate-limiting steps” (the value of the flux control coefficient is never exactly equal to 1). FCC determines the extent to which an increase in a given enzyme activity/concentration can increase the flux through the system.

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DISCLOSURES
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