Possible Factors Determining the Non-Linearity in the $\dot{V}O_2$ –Power Output Relationship in Humans: Theoretical Studies

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Abstract: At low power output exercise (below lactate threshold), the oxygen uptake increases linearly with power output, but at high power output exercise (above lactate threshold) some additional oxygen consumption causes a non-linearity in the overall $\dot{V}O_2$ (oxygen uptake rate)–power output relationship. The functional significance of this phenomenon for human exercise tolerance is very important, but the mechanisms underlying it remain unknown. In the present work, a computer model of oxidative phosphorylation in intact skeletal muscle developed previously is used to examine the background of this relationship in different modes of exercise. Our simulations demonstrate that the non-linearity in the $\dot{V}O_2$ –power output relationship and the difference in the magnitude of this non-linearity between incremental exercise mode and square-wave exercise mode (constant power output exercise) can be generated by introducing into the

model some hypothetical factor F (group of associated factors) that accumulate(s) in time during exercise. The performed computer simulations, based on this assumption, give proper time courses of changes in $\dot{V}O_2$ and [PCr] after an onset of work of different intensities, including the slow component in $\dot{V}O_2$, well matching the experimental results. Moreover, if it is assumed that the exercise terminates because of fatigue when the amount/intensity of F exceed some threshold value, the model allows the generation of a proper shape of the well-known power-duration curve. This fact suggests that the phenomenon of the non-linearity of the $\dot{V}O_2$ –power output relationship and the magnitude of this non-linearity in different modes of exercise is determined by some factor(s) responsible for muscle fatigue. [The Japanese Journal of Physiology 53: 271–280, 2003]

Key words: oxygen uptake kinetics, power output, skeletal muscle, computer model.

The oxygen cost of constant power output exercise performed above the anaerobic (lactate) threshold rises progressively with its duration [1, 2]. This phenomenon, manifesting itself as a progressive increase of oxygen consumption with time, has been called the slow component of oxygen uptake [3]. The extent of the extra oxygen consumption (overconsumption) is positively correlated with the intensity of exercise.

It has been postulated that the presence of the slow component of $\dot{V}O_2$ kinetics implies that the $\dot{V}O_2$ –power output relationship (reflecting the oxygen cost of power production) is non-linear [4]. At low

power outputs, oxygen uptake increases linearly in relation to power output, but at high power outputs, above which a sustained increase in blood lactate concentration occurs (the so-called lactate threshold), an additional increase in $\dot{V}O_2$ above that expected from the extrapolation of this linear relationship, equal up to 20% of the expected value, takes place [4].

Several factors causing the appearance of the slow component of the $\dot{V}O_2$ kinetics and the non-linear increase in $\dot{V}O_2$, which occurs above the lactate threshold, have been proposed in the literature. They include lactate and hydrogen ion accumulation, cate-

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cholamines, increase of muscle temperature, recruitment of type II muscle fibers, proton leak through the inner mitochondrial membrane, and decrease in cytosolic phosphorylation potential (for review, see Whipp [5] and Zoladz and Korzeniewski [6]). Nevertheless, the precise physiological mechanism responsible for these phenomena remains unclear.

At higher exercise intensities, the exercise of muscle is terminated because of fatigue. The time after which a muscle becomes exhausted depends on the exercise intensity: the higher the intensity, the shorter the period of exercise. This dependence is strongly non-linear. It has been described by Wilkie [7, 8] and called the power-duration curve.

The reasons for muscle fatigue are still not well understood. Several factors causing this phenomenon have been proposed: H^+ , NH_3 , IMP, AMP, ADP, P_i , lactate [9], but it is unclear which (if any) contributes to muscle exhaustion and to what extent.

In the present article we demonstrate by means of a computer model of oxidative phosphorylation in muscle developed previously [10] that the phenomenon of the non-linearity in the $\dot{V}O_2$ -power output relationship [4] and its magnitude in different modes of exercise (incremental exercise vs. square-wave exercise) can be generated by introducing into the model some factor F that accumulates with time during exercise with the speed dependent on the exercise intensity. This assumption allows us to obtain a good agreement between computer simulations and a broad set of experimental data, namely, the time courses of changes in $\dot{V}O_2$ and [PCr] after an onset of exercise of different intensities, including the slow component in $\dot{V}O_2$ kinetics. Moreover, it was found that, under the assumption that muscle exercise is terminated because of fatigue when the amount/intensity of F exceed some critical value, the modified model of oxidative phosphorylation involving factor F generates a proper shape of the well-known power-duration curve as described by Wilkie [7, 8]. This unexpected finding prompted us to suggest that the same factor (group of related factors) is responsible both for the phenomenon of muscle fatigue and for the non-linearity in the $\dot{V}O_2$ -power output relationship.

THEORETICAL PROCEDURES

Computer model. In this theoretical study, a dynamic computer model of oxidative phosphorylation in intact skeletal muscle developed previously [10] was used. The following enzymes/processes/metabolic blocks are taken into account explicitly within the model: substrate dehydrogenation (hydro-

gen supply to the respiratory chain), complex I, complex III, complex IV (cytochrome *c* oxidase), proton leak, ATP synthase, ATP/ADP carrier, phosphate carrier, adenylate kinase, creatine kinase, ATP usage. The time variations of the metabolite concentrations that constitute independent variables (NADH, ubiquinol, cytochrome *c*, O_2 , internal protons, internal ATP, internal P_i , external ATP, external ADP, external P_i , PCr) are expressed in the form of a set of ordinary differential equations. The other (dependent) variable values (such as other metabolite concentrations and thermodynamic forces) are calculated from the independent variable values. The set of differential equations is integrated numerically. In each iteration step, new values of rates, concentrations, and other parameters are calculated on the basis of the corresponding values from the previous step. The Gear procedure was used for numerical integration, and the simulation programs were written in the FORTRAN programming language. This original model was used for simulations presented in Fig. 1.

The original model was next slightly modified to involve the factor F that we postulated, responsible for the non-linearity in the $\dot{V}O_2$ -power output relationship. We assumed that F starts to accumulate when the intensity of ATP utilization exceeds some critical value $v_{UT(crit)}$ (corresponding approximately to ATP usage at the lactate threshold), 60 times greater than resting ATP utilization. We assumed the following very simple kinetics of F production:

$$v_F = k_F \cdot (v_{UT} - v_{UT(crit)}) \quad (1)$$

where the rate constant k_F is equal to 4.15×10^{-7} (arbitrary units) and v_{UT} stands for the current rate of ATP utilization. This is probably the simplest possible kinetics of F generation. The k_F constant was adjusted to fit experimental results concerning the extent of the slow component at different exercise intensities. It is also assumed that $v_F = 0$ if $v_{UT} < v_{UT(crit)}$.

Factor F was therefore assumed to cause some futile (not producing power output) ATP utilization besides the normal component of ATP utilization (by, for example, decoupling the actinomyosin-ATPase or increasing a relative contribution of Ca^{2+} -ATPase to ATP usage). This was described within the model in the following way:

$$v_{UT} = k_{UT} / (1 + K_{mATP} / ATP) (1 + F) \quad (2)$$

where 1 in the second brackets corresponds to the normal component of ATP usage and F corresponds to the futile component of ATP usage. We also assume that exercise terminates because of fatigue when the value of F exceeds some critical value F_{crit} equal to

0.22 (arbitrary units). This value was adjusted to cause a termination of exercise because of fatigue after 3 min at the maximal exercise intensity in mode A (see below). When this adjusted value was consequently used in subsequent simulations, it led to a proper prediction of the duration of exercise at different intensities in different exercise modes and allowed the generation of a proper shape of the power-duration curve.

It must be emphasized that F does not necessarily need to be a single physical factor, for example a particular metabolite concentration. Instead it may represent a group of mutually correlated factors, such as $[H^+]$, $[NH_3]$, $[IMP]$, $[AMP]$, $[ADP]$, $[P_i]$, and/or $[lactate]$, the intensities of which increase in parallel in muscle during intensive exercise. The production of F may be increased more directly, e.g., by stimulation of anaerobic glycolysis in different muscle fibers, or more indirectly, e.g., via a recruitment of type II fibers.

We assume that F is decomposed/removed during recovery and therefore that its amount drops to 0 after sufficient recovery following exercise. It is possible to involve in the model a slow decomposition/removal/decay of F (especially during muscle recovery) with the rate linearly proportional to the concentration/intensity of F . For simplicity, we did not do this in our present study.

This slightly modified version of the original model was used for the simulations presented in Figs. 2–4. The complete description of the model used in the present study is given in the APPENDIX.

Simulations. We estimate [10, 12] that in skeletal muscle at intensive exercise the respiration rate is more than 50 times greater than in the resting state, and the concentration of PCr drops two to three times during transition from resting state to intensive work (by intensive work we mean submaximal work in which oxidative ATP production still plays a predominant role). Our simulations show that the above changes can not be explained only by changes in ATP/ADP, the only factor stimulating respiration in isolated mitochondria, and that these changes can be accounted for only if a parallel direct activation by some cytosolic factor(s) of ATP consumption and ATP production (oxidative phosphorylation + substrate dehydrogenation) takes place [10, 12, 13]. We assume that when ATP consumption is activated n times, ATP production is activated $(n-1)^p+1$ times, where $p=0.4$. This assumption satisfactorily agrees with a broad set of experimental data concerning the transition from resting state to intensive exercise in skeletal muscle [11, 12, 14].

Different levels of energy demand (equivalent to the activity of ATP usage) (Figs. 1–4) were established by a multiplication of the rate constant of ATP usage (k_{UT}) in resting state n times, where n was equal to 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, or 120. The rate constants of all oxidative phosphorylation steps but proton leak were multiplied $(n-1)^p+1$ times.

The values of the respiration rate (Figs. 1B, 2B, and 3B) and factor F concentration (intensity) (Figs. 2C and 3C) were recorded in each instance 3 min after a new energy demand was fixed.

The relative power output was assumed to be linearly proportional to the normal component of ATP usage (Eq. 2) minus ATP usage in the resting state, where ATP is used for basic processes (protein synthesis, DNA/RNA synthesis, ion transport) necessary for keeping the cell alive and not for the generation of mechanical work. Both power output and respiration rate were expressed in relative units as a multiplicity

Fig. 1. Simulations of muscle exercise in mode A without fatigue factor F . (A) Time courses of the respiration rate and phosphocreatine concentration. Subsequent relative energy demands that were imposed after each subsequent 3 min of exercise were equal to 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120. (B) Relationship between oxygen consumption (3 min after imposing a new energy demand) and power output.

Fig. 2. Simulations of muscle exercise in mode A with fatigue factor F . (A) Time courses of the respiration rate, phosphocreatine concentration and amount (intensity) of F . Subsequent relative energy demands that were imposed after each subsequent 3 min of exercise were equal to 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120. (B) Relationship between oxygen consumption (3 min after imposing a new energy demand) and power output. (C) Accumulation of factor F (3 min after imposing a new energy demand) at different power outputs.

Fig. 3. Simulations of muscle exercise in mode B with fatigue factor F . (A) Time courses of the respiration rate at different imposed relative energy demands indicated in the figure. (B) Relationship between oxygen consumption (3 min after imposing a given energy demand) and power output. (C) Accumulation of factor F (3 min after imposing a given energy demand) at different power outputs.

Fig. 4. Simulations of the dependence of the exercise duration time until exhaustion on power output. (A) Time courses of oxygen consumption until exhaustion at different power outputs between 10 and 120 (arbitrary units). **(B)** Simulated power-duration curve for the moderate-to-high-power output range.

of the ATP usage and respiration rate in resting state, respectively. One relative unit of power output corresponds to about 3 W, since the relative power output (relative rate constant of ATP usage) equal to 120 (maximal power output in our simulations) corresponds to the maximal power output of about 360 W in our experiments.

Modes of exercise. Two modes of exercise were distinguished and modeled in the present study. In mode A (incremental exercise mode), exercise intensity increases gradually, step-by-step (by an increase in energy demand after every 3 min), during a single exercise mode. In the subsequent 12 steps, energy demand n is increased each time by 10, totally from 10 to 120. In this mode, F that has accumulated at lower-energy-demand stages is transferred to the next higher-energy-demand stages.

In mode B (square-wave exercise), periods of exercise (10 min) of different but constant intensities do

not immediately follow one another, but they are separated by long (enough to recover; the amount of F is simply reset to zero) periods of rest/recovery, during which the system recovers completely. Therefore at the beginning of each simulation, the amount of F is equal to 0. F does not accumulate between subsequent stages with increasing energy demand.

In both modes, it is assumed that when F exceeds F_{crit} , a complete termination of exercise takes place because of the fatigue/exhaustion of muscle.

THEORETICAL RESULTS

First we tried to learn if the slow component of oxygen consumption appears in simulations performed with the aid of our original model of oxidative phosphorylation in muscle [10], which does not involve factor F . As expected, no oxygen overconsumption was observed. This is illustrated in Fig. 1. Figure 1A presents simulated time courses of the respiration rate and [PCr] in mode A. After each increase of energy demand (k_{UT}), the respiration rate quickly reaches a steady state, and its value remains constant until a new higher value of energy demand is imposed. Therefore no slow component is present here. Furthermore, the maximal exercise does not terminate because of fatigue and can potentially last endlessly. Figure 1B presents the simulated dependence of the respiration rate on power output. This relationship is strictly linear.

A quite different situation takes place when fatigue factor F is introduced to our model, as described in THEORETICAL PROCEDURES. The factor F (1) accumulates with time at power outputs above the lactate threshold (Eq. 1) and (2) causes some additional futile ATP usage not associated with power production (Eq. 2). Figure 2 presents the results of simulations that are fully analogous to the simulations shown in Fig. 1, but they involve factor F . Figure 2A presents simulated time courses of the respiration rate, [PCr] and the amount of F in mode A with F . It can be seen that for lower exercise intensities the behavior of the system is identical in both instances (with and without F). However, at higher energy demands the slow component of oxygen consumption appears when F is present. The system is unable to reach a steady state after subsequent increases of energy demand. The increase in the respiration rate in time is faster at higher energy demands. The same property concerns the decrease of phosphocreatine concentration in time. F accumulates in a near-linear manner at each given energy demand; its rate of accumulation increases, however, with the increase in energy demand. Ultimately the exercise

terminates because of fatigue, when the amount of F exceeds the critical value F_{crit} . These theoretical results agree well with experiments involving mode A of exercise [4].

Figure 2B presents the simulated dependence of the respiration rate on power output in mode A with F . It can easily be seen that extra oxygen consumption appears at higher power outputs (in relation to the oxygen consumption expected from a linear extrapolation of the dependence of oxygen consumption on power output at lower power outputs). This oxygen overconsumption reaches a maximum of 19% at the highest exercise intensity, which agrees well with experimental results concerning mode A [4]. Figure 2C presents the simulated accumulation of F at different power outputs. The amount of F depends on power output in a near-exponential manner, as shown, until F exceeds F_{crit} and exercise terminates.

To determine whether and how the size of the slow component and the accumulation of F depend on the mode of exercise, we simulated muscle exercise in mode B, using the same parameter values as in the simulations in mode A, shown in Fig. 2. The theoretical results obtained are presented in Fig. 3. Figure 3A presents the simulated time courses of the respiration rate at different energy demands fixed from the beginning to the end of each simulation. One can see that at lower energy demands a steady state is quickly reached and the oxygen consumption remains constant in time. At higher energy demands the slow component appears; however, the increase in the respiration rate here is much slower than in mode A at the same demands. Moreover, exercise does not terminate after 3 min at the highest energy demand, as in Fig. 2A (mode A). It terminates after 11 min, outside the diagram. Figure 3B, representing the simulated dependence between the oxygen consumption and power output in mode B, shows that the oxygen overconsumption is much smaller in mode B than in mode A, reaching a maximum of only 6%. The reason for this is presented in Fig. 3C—the accumulation of the fatigue factor F is much smaller in mode B than in mode A at the same power outputs. This is because in mode B, each subsequent simulation for a new higher energy demand starts from the amount of F equal to 0, but in mode A each new step with higher energy demand starts with the amount of F that has accumulated in previous steps. These theoretical results agree well with our preliminary experimental results (data not published); we observed that the extra oxygen consumption (measured 3 min after the onset of exercise of a given intensity) was equal to 5.5% in square-wave exercise, in which periods of constant-intensity

exercise lasting 6 min were separated by 6 min—lasting periods of recovery, and it amounted to 14% in incremental-mode exercise (increase of power output by 30 W every 3 min) for submaximal power output (250 W) (the two modes of exercise were performed by the same group of subjects). Furthermore, our model explains well our other experimental finding [15], that oxygen overconsumption is significantly smaller when power output is increased by 15 W every min than when power output is increased by 30 W every 3 min. The difference is because F had less time to accumulate in the former instance (not shown).

We studied in a theoretical way the time course of oxygen consumption at different constant exercise intensities during a long period (50 min). In particular, we examined the duration time of exercise until it terminates because of fatigue ($F > F_{\text{crit}}$). The results of computer simulations are presented in Fig. 4A. It may be seen that the slow component appears above the lactate threshold and that its magnitude (steepness of rise in oxygen consumption) increases with power output. This is accompanied by a decrease in the duration time of constant power output exercise. Figure 4B presents the simulated relationship between the duration of exercise (sustainable until exhaustion) and power output, extracted from the data presented in Fig. 4A. The dependence is strongly nonlinear (near-exponential), and it is similar to the experimental power-duration curve [7, 8, 16].

DISCUSSION

The slow component of $\dot{V}O_2$ kinetics during a constant power output exercise [3, 5], and the non-linear increase in $\dot{V}O_2$ occurring at high work intensities during incremental exercise test [4] illustrates that exceeding a given power output causes a non-proportional rise in $\dot{V}O_2$ in relation to the mechanical power output. This drop in mechanical efficiency starts at power outputs above the lactate threshold and increases with the increase of power output. Several factors, including lactate and hydrogen ion accumulation, catecholamines, increase of muscle temperature, recruitment of type II muscle fibers, proton leak through the inner mitochondrial membrane, and decrease in cytosolic phosphorylation potential, have been suggested as possible causes of the slow component of oxygen consumption (for review, see Whipp [5] and Zoladz and Korzeniewski [6]); however, the physiological mechanism responsible for this phenomenon remains unknown.

In the present article we postulate that some factor F that accumulates during intensive exercise may be

responsible for the slow component of oxygen consumption and the non-linearity in the VO₂-power output relationship. We show that the original version of our model of oxidative phosphorylation in muscle [10], not involving F , is unable to generate the slow component phenomenon (Fig. 1A) and the non-linearity in the VO₂-power output relationship (Fig. 1B). Therefore we introduced factor F to the modified version of the model and used this version for further theoretical studies. Two exercise modes were distinguished and modeled. In mode A, exercise intensity increases gradually, step-by-step (incremental exercise mode) during a single exercise mode. In mode B, periods of exercise of different but constant intensities do not immediately follow one another, but are separated by long periods of rest/recovery, during which the system recovers completely (square-wave mode). In both modes, it is assumed that the extent of the slow component is proportional to F , and that when F exceeds F_{crit} , a complete termination of exercise takes place because of the fatigue/exhaustion of muscle.

The performed simulations agree well with experimental data concerning the exercise in mode A (and with our unpublished data concerning mode B). For example, they predict that the extent of the slow component increases with power output. This agreement is partly due to adjusting appropriate parameter values (k_F , F_{crit}). On the other hand, the model produces several predictions that cannot be easily made without modeling. Our computer simulations suggest that exercise terminates faster in mode A than in mode B because of muscle fatigue at the highest power output. Furthermore, the model predicts that the extent of the slow component (oxygen overconsumption) is much greater in mode A than in mode B. These predictions can be tested/confirmed experimentally, and we plan to continue our experimental studies concerning this problem.

However, and more important, our assumptions concerning the kinetic properties of the factor F that were primarily aimed to explain the slow component phenomenon unexpectedly allowed the proper shape of the power-duration curve [7, 8], presented in Fig. 4B, to be generated. It is theoretically possible that one simple theoretical assumption can explain two completely different complex phenomena by pure chance, but this does not seem likely. Therefore our theoretical results suggest a strict causal relationship between the slow component and non-linearity in the VO₂-power output relationship and muscle fatigue.

Our model makes several predictions that can help to identify the physical nature of the postulated fatigue factor F . First, the accumulation of F depends in

a near-exponential manner on power output. Second, the amount/intensity of F at the same high power output is greater in mode A than in mode B. Third, the slow component depends nearly linearly on F . Fourth, high-intensity exercise always terminates when F exceeds F_{crit} . These predictions can, at least in principle, be tested experimentally for each candidate for factor F . The factor that exhibits these properties will most probably be identical with (or at least closely related to) factor F .

The question arises in what way factor F causes the oxygen overconsumption during high-power output exercise. We postulate that the side of energy utilization but not production, is responsible for the non-linearity in the VO₂-power output relationship [6]. Consequently, we assume in the present article that ATP consumption and not ATP production is decoupled by F . This assumption is supported by the work of Tonkonogi *et al.* [17] in which the authors found no effect of fatigue on mitochondria efficiency after 30 min of intensive (well above the lactate threshold) exercise. However, in principle, a decoupling of ATP production by F would give results similar to a decoupling of ATP consumption.

It must be emphasized that the factor F we postulate can be responsible for fatigue only in high-intensity exercise (above the lactate threshold) and not in long-term low-intensity exercise with power outputs below the lactate threshold. On the other hand, our model involves only oxidative ATP production and therefore cannot be applied to short-term maximal-intensity exercise, where glycolytic ATP supply plays an important role or even predominates. Therefore the maximal oxygen uptake and power output in Fig. 4 correspond to the maximal capacity of oxidative phosphorylation for oxygen consumption and ATP synthesis *in vivo*.

We postulate that some factor (or a group of mutually associated factors) that causes fatigue during intensive muscle exercise is involved in the origin of the slow component in oxygen uptake kinetics and of the non-linearity in the VO₂-power output relationship.

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REFERENCE

1. Costill DL: Metabolic responses during distance running. *J Appl Physiol* 28: 251–255, 1970
2. Hagberg JM, Mullin JP, and Nagle FJ: Oxygen consumption during constant-load exercise. *J Appl Physiol* 45: 381–384, 1978
3. Whipp BJ and Wasserman K: Oxygen uptake kinetics for various intensities of constant load work. *J Appl*

- Physiol 33: 351–356, 1972
4. Zoladz JA, Rademaker ACHJ, and Sargeant AJ: Non-linear relationship between O_2 uptake and power output at high intensities of exercise in humans. *J Physiol (Lond)* 488: 211–217, 1995
 5. Whipp BJ: The slow component of O_2 uptake kinetics during heavy exercise. *Med Sci Sports Exerc* 26: 1319–1326, 1994
 6. Zoladz JA and Korzeniewski B: Physiological background of the change point in $\dot{V}O_2$ and the slow component of oxygen uptake kinetics. *J Physiol Pharmacol* 52: 167–184, 2001
 7. Wilkie DR: Man as a source of mechanical power. *Ergonomics* 3: 1–8, 1960
 8. Wilkie DR: Equations describing power input by human as function of duration of exercise. In: *Exercise Bioenergetics and Gas Exchange*, ed. Cerretelli P and Whipp BJ, Elsevier, Amsterdam, pp 75–80, 1981
 9. Turner DL and Hoppeler H: Muscle energetics: aerobic strategies. In: *Physiological Determinants of Exercise Tolerance in Humans*. Studies in Physiology, ed. Whipp BJ and Sargeant AJ, Portland Press, London, pp 29–47, 1999
 10. Korzeniewski B and Zoladz JA: A model of oxidative phosphorylation in skeletal muscle. *Biophys Chem* 92: 17–34, 2001
 11. Hochachka PW: *Muscles as Molecular and Metabolic Machines*, CRC Press, Boca Raton, 1994
 12. Korzeniewski B: Regulation of ATP supply in mammalian skeletal muscle during resting state→intensive work transition. *Biophys Chem* 83: 19–34, 2000
 13. Korzeniewski B: Regulation of ATP supply during muscle contraction: theoretical studies. *Biochem J* 330: 1189–1195, 1998
 14. Jeneson JAL, Wiseman RW, Westerhoff HV, and Kushmerick MJ: The signal transduction function for oxidative phosphorylation is at least second order in ADP. *J Biol Chem* 271: 27995–27998, 1996
 15. Zoladz JA, Duda K, and Majerczak J: Oxygen uptake-power output relationship during incremental exercise test in humans. *J Physiol (Lond)* 511P: 8P, 1998
 16. Fukuba Y and Whipp BJ: A metabolic limit on the ability to make up for lost time in endurance events. *J Appl Physiol* 87: 853–861, 1999
 17. Tonkonogi M, Harris B, and Sahlin K: Mitochondrial oxidative function in human saponin-skinned muscle fibres: effects of prolonged exercise. *J Physiol (Lond)* 510: 279–286, 1998

APPENDIX

Concise complete kinetic description of the dynamic model of oxidative phosphorylation in intact skeletal muscle with the F factor was used in the present study.

Subscripts: e, external (cytosolic); i, internal (mitochondrial); t, total; f, free; m, magnesium complex; j, monovalent.

Kinetic equations

All reaction rates are expressed in $\mu\text{M min}^{-1}$.

Substrate dehydrogenation:

$$v_{\text{DH}} = k_{\text{DH}} [1 / \{1 + K_{\text{mN}} / (\text{NAD}^+ / \text{NADH})\}^{p_{\text{D}}}]$$

$$k_{\text{DH}} = 28,074 \mu\text{M min}^{-1}, K_{\text{mN}} = 100, p_{\text{D}} = 0.8.$$

Complex I:

$$v_{\text{CI}} = k_{\text{CI}} \cdot \Delta G_{\text{CI}}$$

$$k_{\text{CI}} = 238.95 \mu\text{M mV}^{-1} \text{ min}^{-1}.$$

Complex III:

$$v_{\text{C3}} = k_{\text{C3}} \cdot \Delta G_{\text{C3}}$$

$$k_{\text{C3}} = 136.41 \mu\text{M mV}^{-1} \text{ min}^{-1}$$

Complex IV:

$$v_{\text{C4}} = k_{\text{C4}} \cdot a^{2+} \cdot c^{2+} \{1 / (1 + K_{\text{mO}} / O_2)\}$$

$$k_{\text{C4}} = 3.600 \mu\text{M}^{-1} \text{ min}^{-1}, K_{\text{mO}} = 120 \mu\text{M} \quad (\text{apparent } K_{\text{mO}} = 0.8 \mu\text{M}).$$

ATP synthase:

$$v_{\text{SN}} = k_{\text{SN}} (\gamma - 1) / (\gamma + 1)$$

$$k_{\text{SN}} = 34,316 \mu\text{M min}^{-1}, \gamma = 10^{\Delta G_{\text{SN}} / Z}.$$

ATP/ADP carrier:

$$v_{\text{EX}} = k_{\text{EX}}$$

$$\left(\frac{\text{ADP}_{\text{fe}}}{\text{ADP}_{\text{fe}} + \text{ATP}_{\text{fe}} \cdot 10^{-\Psi_{\text{e}}/Z}} - \frac{\text{ADP}_{\text{fi}}}{\text{ADP}_{\text{fi}} + \text{ATP}_{\text{fi}} \cdot 10^{-\Psi_{\text{i}}/Z}} \right) \cdot \left(\frac{1}{1 + K_{\text{mADP}} / \text{ADP}_{\text{fe}}} \right)$$

$$k_{\text{EX}} = 54,572 \mu\text{M min}^{-1}, K_{\text{mADP}} = 3.5 \mu\text{M}.$$

Phosphate carrier:

$$v_{\text{PI}} = k_{\text{PI}} \cdot (\text{Pi}_{\text{je}} \cdot H_{\text{e}} - \text{Pi}_{\text{ji}} \cdot H_{\text{i}})$$

$$k_{\text{PI}} = 69.421 \mu\text{M}^{-1} \text{ min}^{-1}$$

ATP usage:

$$v_{\text{UT}} = k_{\text{UT}} \{1 / (1 + K_{\text{mA}} / \text{ATP}_{\text{te}})\} \cdot (1 + F)$$

$k_{\text{UT}} = 686.50 \mu\text{M min}^{-1}$ (resting state) or $k_{\text{UT}} =$ multiplicity of this value (active state), as indicated in the text and figure legends, $K_{\text{mA}} = 150 \mu\text{M}$.

Proton leak:

$$v_{\text{LK}} = k_{\text{LK1}} \cdot \{\exp(k_{\text{LK2}} \cdot \Delta p) - 1\}$$

$$k_{\text{LK1}} = 2.500 \mu\text{M min}^{-1}, k_{\text{LK2}} = 0.038 \text{ mV}^{-1}.$$

Adenylate kinase:

$$v_{\text{AK}} = k_{\text{fAK}} \cdot \text{ADP}_{\text{fe}} \cdot \text{ADP}_{\text{me}} - k_{\text{bAK}} \cdot \text{ATP}_{\text{me}} \cdot \text{AMP}_{\text{e}}$$

$$k_{\text{fAK}} = 862.10 \mu\text{M}^{-1} \text{ min}^{-1}, k_{\text{bAK}} = 22.747 \mu\text{M}^{-1} \text{ min}^{-1}.$$

Creatine kinase:

$$v_{CK} = k_{fCK} \cdot ADP_{te} \cdot PCr \cdot H_e^+ - k_{bCK} \cdot ATP_{te} \cdot Cr$$

$$k_{fCK} = 1.9258 \mu M^{-2} \min^{-1}, \quad k_{bCK} = 0.00087538 \mu M^{-1} \min^{-1}.$$

Proton efflux:

$$v_{EFF} = k_{EFF} \cdot (pH_0 - pH_e)$$

$$k_{EFF} = 10,000 \mu M \min^{-1}, \quad pH_0 = 7.0.$$

Production of F :

$$v_F = k_F \cdot (v_{UT} - v_{UT(crit)}) \quad \text{if } v_{UT} > v_{UT(crit)}$$

or

$$v_F = 0 \quad \text{if } v_{UT} < v_{UT(crit)}$$

$$k_F = 4.15 \times 10^{-7} \min^{-1}; \quad \text{initial } F = 0.$$

Set of differential equations

$$NADH = (v_{DH} - v_{C1}) \cdot R_{cm} / B_N$$

$$UQH_2 = (v_{C1} - v_{C3}) \cdot R_{cm}$$

$$c^{2+} = (v_{C3} - 2v_{C4}) \cdot 2R_{cm}$$

$$O_2 = 0 \quad (\text{constant saturated oxygen concentration} \\ = 240 \mu M) \quad \text{or}$$

$$O_2 = -v_{C4}$$

$$H_i^+ = -(2 \times (2 + 2u) \cdot v_{C4} + (4 - 2u) \cdot v_{C3} + 4v_{C1} - n_A \cdot v_{SN} \\ - u \cdot v_{EX} - (1 - u) \cdot v_{PI} - v_{LK}) \cdot R_{cm} / r_{buffi}$$

$$ATP_{ti} = (v_{SN} - v_{EX}) \cdot R_{cm}$$

$$Pi_{ti} = (v_{PI} - v_{SN}) \cdot R_{cm}$$

$$ATP_{te} = v_{EX} - v_{UT} + v_{AK} + v_{CK}$$

$$ADP_{te} = v_{UT} - v_{EX} - 2v_{AK} - v_{CK}$$

$$Pi_{te} = v_{UT} - v_{PI}$$

$$PCr = -v_{CK}$$

$$H_e^+ = 2 \times (2 + 2u) \cdot v_{C4} + (4 - 2u) \cdot v_{C3} + 4v_{C1} - n_A \cdot v_{SN} \\ - u \cdot v_{EX} - (1 - u) \cdot v_{PI} - v_{LK} - v_{CK} - v_{EFF} / r_{buffe}$$

$$F = v_F; \quad \text{initial } F = 0$$

$$R_{cm} = 15 \quad (\text{cell volume/mitochondria volume ratio}), \\ B_N = 5 \quad (\text{buffering capacity coefficient for NAD}).$$

Calculations

$$c^{3+} = c_t - c^{2+}$$

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

$$UQ = U_t - UQH_2$$

$$U_t = 1,350 \mu M \quad (= UQH_2 + UQ, \text{ total concentration of ubiquinone}).$$

$$NAD^+ = N_t - NADH$$

$$N_t = 2,970 \mu M \quad (= NADH + NAD^+, \text{ total concentration of NAD}).$$

$$AMP_e = A_{eSUM} - ATP_{te} - ADP_{te}$$

$$A_{eSUM} = 6700.2 \mu M \quad (= ATP_{te} + ADP_{te} + AMP_e, \text{ total external adenine nucleotide concentration}),$$

$$ADP_{ti} = A_{iSUM} - ATP_{ti}$$

$$A_{iSUM} = 16,260 \mu M \quad (= ATP_{ti} + ADP_{ti}, \text{ total internal adenine nucleotide concentration}).$$

$$Cr = C_{SUM} - PCr$$

$$C_{SUM} = 35,000 \mu M \quad (= Cr + PCr, \text{ total creatine concentration}).$$

$$P_{SUM} = 55,659 \mu M \quad (= PCr + 3ATP_{te} + 2ADP_{te} + AMP_e \\ + Pi_{te} + (3ATP_{ti} + 2ADP_{ti} + Pi_{ti}) / R_{cm}, \\ \text{total phosphate pool})$$

$$Mg_{fe} = 4,000 \mu M \quad (\text{free external magnesium concentration}).$$

$$ATP_{fe} = ATP_{te} / (1 + Mg_{fe} / k_{DTe})$$

$$k_{DTe} = 24 \mu M \quad (\text{magnesium dissociation constant for external ADP}).$$

$$ATP_{me} = ATP_{te} - ATP_{fe} \quad ADP_{fe} = ADP_{te} / (1 + Mg_{fe} / k_{DDe})$$

$$k_{DDe} = 347 \mu M \quad (\text{magnesium dissociation constant for external ATP}).$$

$$ADP_{me} = ADP_{te} - ADP_{fe}$$

$$Mg_{fi} = 380 \mu M \quad (\text{free internal magnesium concentration}).$$

$$ATP_{fi} = ATP_{ti} / (1 + Mg_{fi} / k_{DTi})$$

$$k_{DTi} = 17 \mu M \quad (\text{magnesium dissociation constant for internal ATP}).$$

$$ATP_{mi} = ATP_{ti} - ATP_{fi}$$

$$ADP_{fi} = ADP_{ti} / (1 + Mg_{fi} / k_{DDi})$$

$$k_{DDi} = 282 \mu M \quad (\text{magnesium dissociation constant for internal ADP}).$$

$$ADP_{mi} = ADP_{ti} - ADP_{fi}$$

$$T = 298, \quad R = 0.0083 \text{ kJ mol}^{-1} \text{ K}^{-1}, \quad F = 0.0965 \text{ kJ mol}^{-1} \text{ mV}^{-1}, \\ S = 2.303 \times R \times T, \quad Z = 2.303 \times R \times T / F, \quad u = 0.861 \\ (= \Delta \Psi / \Delta p), \quad pH_e = 6.9 \quad (= \text{constant}).$$

$$pH_i = -\log(H_i / 1,000,000) \quad (H_i \text{ expressed in } \mu M)$$

$$\Delta pH = Z(pH_i - pH_e)$$

$$\Delta p = 1 / (1 - u) \Delta pH$$

$$\Delta \Psi = -(\Delta p - \Delta \text{pH})$$

$$\Psi_i = 0.65 \times \Delta \Psi$$

$$\Psi_e = -0.35 \times \Delta \Psi$$

$$c_{0i} = (10^{-\text{pHi}} - 10^{-\text{pHi} - \text{dpH}}) / \text{dpH}$$

("natural" buffering capacity for H⁺ in matrix)

$$\text{dpH} = 0.001.$$

$$r_{\text{buffi}} = c_{\text{buffi}} / c_{0i}$$

(buffering capacity coefficient for H⁺ in matrix)

$$c_{\text{buffi}} = 0.022 \text{ M H}^+ / \text{pH unit (buffering capacity for H}^+ \text{ in matrix).}$$

$$c_{0e} = (10^{-\text{pHe}} - 10^{-\text{pHe} - \text{dpH}}) / \text{dpH}$$

("natural" buffering capacity for H⁺ in cytosol)

$$\text{dpH} = 0.001.$$

$$r_{\text{buffe}} = c_{\text{buffe}} / c_{0e} \text{ (buffering capacity coefficient for H}^+ \text{ in cytosol)}$$

$$c_{\text{buffe}} = 0.025 \text{ M H}^+ / \text{pH unit (buffering capacity for H}^+ \text{ in cytosol).}$$

$$\text{Pi}_{\text{je}} = \text{Pi}_{\text{te}} / (1 + 10^{\text{pHe} - \text{pKa}})$$

$$\text{Pi}_{\text{ji}} = \text{Pi}_{\text{ti}} / (1 + 10^{\text{pHi} - \text{pKa}})$$

$$\text{pKa} = 6.8.$$

$$\Delta G_{\text{SN}} = n_A \times \Delta p - \Delta G_{\text{P}} \text{ (thermodynamic span of ATP synthase)}$$

$$\Delta G_{\text{P}} = \Delta G_{\text{P0}} / F + Z \times \log(1,000,000 \times \text{ATP}_{\text{ti}} / (\text{ADP}_{\text{ti}} \times \text{Pi}_{\text{ti}})) \text{ (concentrations expressed in } \mu\text{M)}$$

$$n_A = 2.5 \text{ (phenomenological H}^+ \text{ / ATP stoichiometry of ATP synthase), } \Delta G_{\text{P0}} = 31.9 \text{ kJ mol}^{-1}.$$

$$E_{\text{mN}} = E_{\text{mN0}} + Z/2 \times \log(\text{NAD}^+ / \text{NADH})$$

(NAD redox potential)

$$E_{\text{mN0}} = -320 \text{ mV.}$$

$$E_{\text{mU}} = E_{\text{mU0}} + Z/2 \times \log(\text{UQ} / \text{UQH}_2)$$

(ubiquinone redox potential)

$$E_{\text{mU0}} = 85 \text{ mV.}$$

$$E_{\text{mc}} = E_{\text{mc0}} + Z \times \log(c^{3+} / c^{2+})$$

(cytochrome *c* redox potential)

$$E_{\text{mc0}} = 250 \text{ mV.}$$

$$E_{\text{ma}} = E_{\text{mc}} + \Delta p \times (2 + 2u) / 2$$

(cytochrome *a*₃ redox potential)

$$A_{3/2} = 10^{(E_{\text{ma}} - E_{\text{ma0}}) / Z} \text{ (} a^{3+} / a^{2+} \text{ ratio)}$$

$$a^{2+} = a_{\text{t}} / (1 + A_{3/2})$$

(concentration of reduced cytochrome *a*₃)

$$a^{3+} = a_{\text{t}} - a^{2+}$$

$$a_{\text{t}} = 135 \mu\text{M}$$

$$E_{\text{ma0}} = 540 \text{ mV.}$$

$$\Delta G_{\text{C1}} = E_{\text{mU}} - E_{\text{mN}} - \Delta p \times 4/2$$

(thermodynamic span of complex I)

$$\Delta G_{\text{C3}} = E_{\text{mc}} - E_{\text{mU}} - \Delta p \times (4 - 2u) / 2$$

(thermodynamic span of complex III)