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Research Article

# Skeletal muscle biochemical origin of exercise intensity domains and their relation to whole-body $\dot{V}O_2$ kinetics

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This article presents the biochemical intra-skeletal-muscle basis of exercise intensity domains: moderate (M), heavy (H), very heavy (VH) and severe (S). Threshold origins are mediated by a '$P_i$ double-threshold' mechanism of muscle fatigue, which assumes (1) additional ATP usage, underlying muscle $\dot{V}O_2$ and metabolite slow components, is initiated when inorganic phosphate ($P_i$) exceeds a critical value ($Pi_{crit}$); (2) exercise is terminated because of fatigue, when $P_i$ reaches a peak value ($Pi_{peak}$); and (3) the $P_i$ increase and additional ATP usage increase mutually stimulate each other forming a positive feedback. M/H and H/VH borders are defined by $P_i$ on-kinetics in relation to $Pi_{crit}$ and $Pi_{peak}$. The values of the ATP usage activity, proportional to power output (PO), for the M/H, H/VH and VH/S borders are lowest in untrained muscle and highest in well-trained muscle. The metabolic range between the M/H and H/VH border (or 'H space') decreases with muscle training, while the difference between the H/VH and VH/S border (or 'VH space') is only weakly dependent on training status. The absolute magnitude of the muscle $\dot{V}O_2$ slow-component, absent in M exercise, rises gradually with PO to a maximal value in H exercise, and then decreases with PO in VH and S exercise. Simulations of untrained, physically active and well-trained muscle demonstrate that the muscle M/H border need not be identical to the whole-body M/H border determined from pulmonary $\dot{V}O_2$ on-kinetics and blood lactate, while suggesting that the biochemical origins of the H/VH border reside within skeletal muscle and correspond to whole-body critical power.

## Introduction

Skeletal muscle metabolic flux (flow of metabolites through the bioenergetic system, especially ATP turnover) can vary over 100-fold between resting and maximal exercise conditions. This continuous spectrum is divided into several ranges, or intensity domains, that differ qualitatively by their biochemical and kinetic behaviors. Three main classifications of exercise intensity domains in humans have been postulated in the literature (see [1,2] for review), which are determined from whole-body responses, typically in terms of pulmonary $\dot{V}O_2$ and blood lactate on-kinetics. The simplest system involves moderate (M), heavy (H) and severe (S) intensity domains.

The M exercise intensity domain is located below the lactate threshold (LT) or gas exchange threshold (GET), and the pulmonary $\dot{V}O_2$ on-kinetics comprise only cardiodynamic (phase I) and fundamental (phase II) components. In the muscle, M exercise is also characterized by an initial delay ('lag phase') in the $\dot{V}O_2$ on-kinetics, analogous, but mechanistically distinct, to phase I, and fundamental (phase II) components. Muscle and pulmonary phase II $\dot{V}O_2$ on-kinetics increase approximately exponentially and reach a steady-state (plateau) after approximately 2–3 min. No slow component of the $\dot{V}O_2$ on-kinetics is present.

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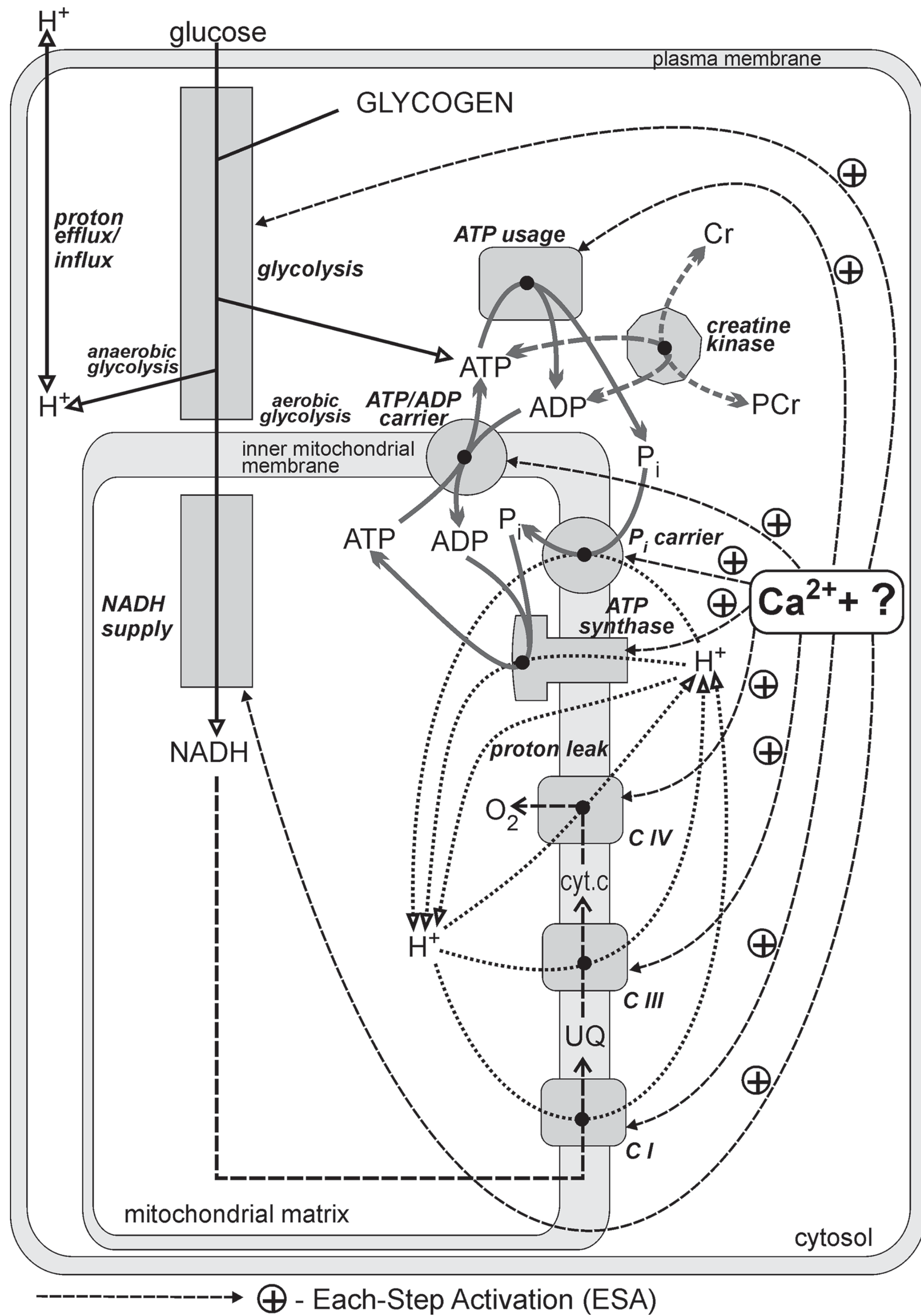


**Figure 1. Simplified scheme of the bioenergetic system in the skeletal muscle cell**

The elements of the system that are explicitly taken into account within the computer model used are shown. Essentially, all elements of the system are directly activated by some mechanism involving cytosolic $Ca^{2+}$ (inner mitochondrial membrane OXPHOS complexes, malate-aspartate shuttle and glycolysis) and mitochondrial $Ca^{2+}$ (NADH supply). The question mark ('?') indicates some still undetermined factor/mechanism cooperating with $Ca^{2+}$, for instance calmodulin-like protein 'presenting' $Ca^{2+}$ to enzymes/carriers and/or protein phosphorylation. CI, CIII, CIV, complexes I, III and IV of the respiratory chain, respectively; cyt.c, cytochrome c; UQ, ubiquinone.



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Blood lactate ($L^-$) initially increases slightly above the resting level but returns to or below the resting value after a few minutes. In the steady state of M exercise, pulmonary $\dot{V}CO_2/\dot{V}O_2$ stabilizes at or below 1.0 [1–3].

The H exercise intensity domain comprises power outputs (POs) and metabolic fluxes (including $\dot{V}O_2$) between LT/GET and critical power (CP, the asymptote of the power–duration curve, see [4,5]). Here, the slow component of the $\dot{V}O_2$ on-kinetics appears 1.5–2 min after the onset of exercise, which is superimposed on the primary phase II on-kinetics. However, after some time (typically, 15–20 min) $\dot{V}O_2$ stabilizes at a level below $\dot{V}O_{2max}$, but above the level expected from phase II on-kinetics and exercise can be well sustained. Blood lactate ($L^-$) also initially rises and then stabilizes at a value above resting. $\dot{V}CO_2/\dot{V}O_2$ transiently increases and slowly declines over ~15–20 min to a value approximately equal to 1.0.

Finally, the S exercise intensity domain is characterized by the presence of a $\dot{V}O_2$ on-kinetics slow component that is not able to stabilize, causing $\dot{V}O_2$ to increase continuously until exercise is voluntarily terminated, or when it reaches $\dot{V}O_{2max}$. S intensity exercise is associated with progressive loss of efficiency related to fatigue, which continues until termination or intolerance. Blood $L^-$ increases continuously throughout S intensity exercise and $\dot{V}CO_2/\dot{V}O_2$ increases abruptly followed by a slow decline but without achieving stability and remains above 1.0 at termination.

Two other exercise intensity domain classification schemes constitute a modification of the above classification. Some define extreme (E) exercise intensity from the greatest PO for which $\dot{V}O_2$ is able to reach $\dot{V}O_{2max}$; exercise in the E domain is therefore characterized by task failure from fatigue prior to reaching $\dot{V}O_{2max}$ [6]. E intensity exercise is typically limited to less than approximately 2 min in duration. Another modification was proposed by Whipp [1,3,7], who split the S exercise domain into very heavy (VH) and severe (S) on the basis of whether or not the primary component of the $\dot{V}O_2$ on-kinetics is predicted to project below (VH domain) or above (S domain) $\dot{V}O_{2max}$.

The mechanism(s) that cause exercise termination at (or below) $\dot{V}O_{2max}$ remain uncertain [8]. Muscle fatigue can lead to termination of exercise [9,10]. Fatigue is related to a fall in the efficiency of the skeletal muscle bioenergetic system [11]. Recently, the '$P_i$ double-threshold' mechanism of muscle fatigue has been proposed to help explain observed bioenergetics system behaviors [12,13]. This mechanism is based on three assumptions: (1) the additional ATP usage, which underlies the slow component of $\dot{V}O_2$ and metabolite on-kinetics, is initiated when $P_i$ exceeds a certain critical value, termed $Pi_{crit}$ [12]; (2) muscle work is terminated because of fatigue when $P_i$ reaches another, higher, peak value ($Pi_{peak}$) [14]; and (3) $P_i$ increase and additional ATP usage increase mutually stimulate each other, thus forming a self-driving positive feedback mechanism [12]. This latter assumption ultimately causes $P_i$ to reach $Pi_{peak}$ (and $\dot{V}O_2$ to reach $\dot{V}O_{2max}$) and exercise termination because of fatigue. This mechanism is able to generate many various, apparently unrelated, muscle system properties: changes over time of several variables including muscle $\dot{V}O_2$, cytosolic ADP, pH, PCr and $P_i$ during rest-to-work transition in skeletal muscle; the end-exercise constancy of these variables at different power outputs above CP; the hyperbolic shape of the power–duration curve with CP as an asymptote; and the hypoxia/hyperoxia-induced decrease/increase in CP and $\dot{V}O_{2max}$, and increase/decrease of $t_{0.63}$ [12].

In addition, the '$P_i$ double-threshold' mechanism is able to account for training-induced changes in $\dot{V}O_{2max}$, CP and $\dot{V}O_2$ on-kinetics (shortening of $t_{0.63}$, decrease of the slow component), provided that muscle training causes an increase in OXPHOS activity and decrease in $Pi_{peak}$ [13]. The '$P_i$ double-threshold' mechanism is also able to account for observed effects on muscle bioenergetic responses and exercise tolerance in patients with mitochondrial and nuclear DNA mutations causing deficiencies in OXPHOS [15] and the effect of training in such patients [16].

This theoretical study aims to identify intramuscular origins of whole-body exercise intensity domains. We use the intensity domain terminology of [3] (i.e. M, H, VH and S) to define exercise intensity domains at the skeletal muscle metabolism level and to relate these to intensity domains defined at the whole-body level in terms of pulmonary $\dot{V}O_2$ and blood $L^-$ kinetics. In other words, we aim to link skeletal muscle biochemical/molecular events to physiologic responses during rest-to-work transitions and development of muscle fatigue. We define the muscle exercise intensity domains in terms of the '$P_i$ double-threshold' mechanism of muscle fatigue, involving the $P_i$ on-kinetics, $Pi_{crit}$ and $Pi_{peak}$, and postulate that whole-body exercise intensity domains originate primarily at the molecular level. As events defining whole-body intensity domains are influenced by extra-muscular events (e.g. blood flow distribution, lactate clearance and oxygen consumption by tissues other than working muscles), we investigate whether borders between the M and H domains are similar at the muscle and whole-body levels.

# Theoretical results

The scheme of the bioenergetic system, showing the elements accounted for explicitly within the model used in this study, is presented in Figure 1.

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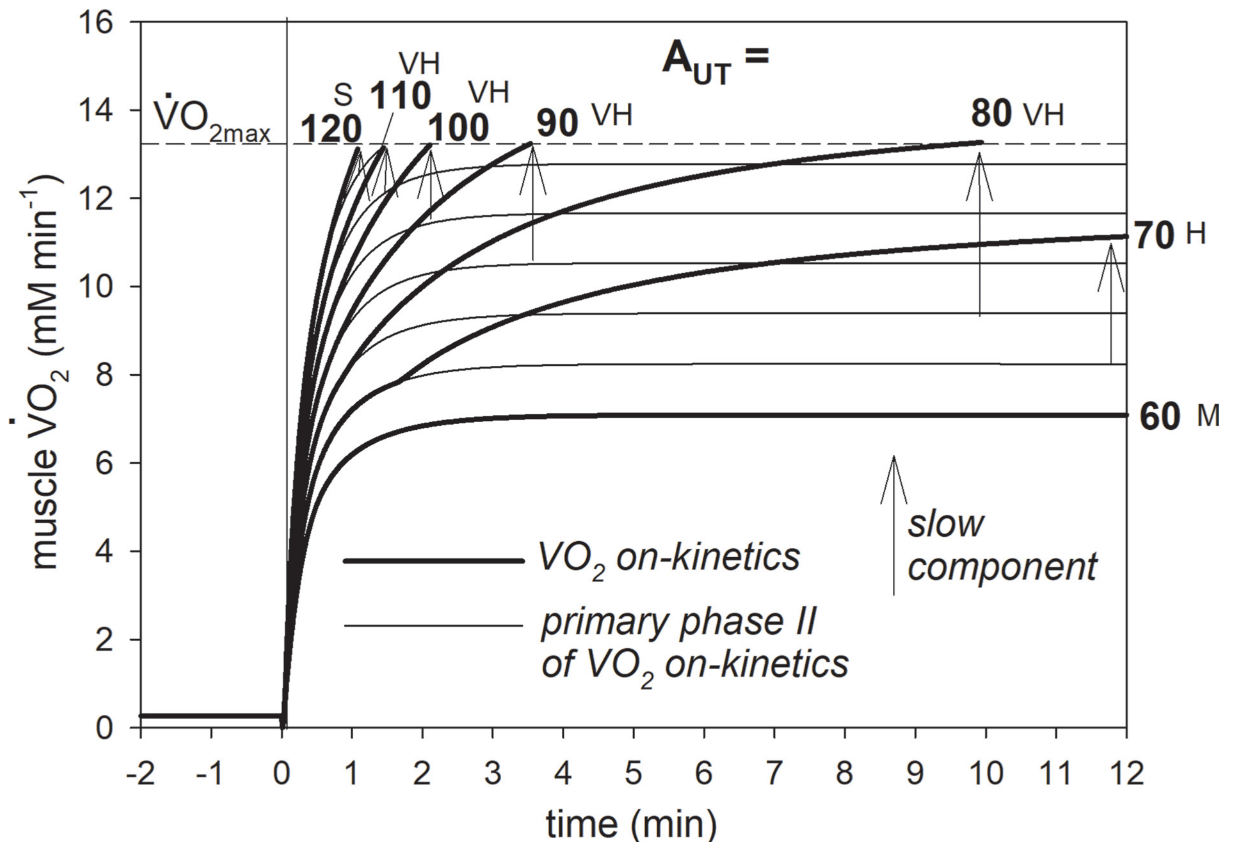


**Figure 2. Simulated muscle $\dot{V}O_2$ on-kinetics at different ATP usage activities ($A_{UT}$, proportional to PO)**

Different exercise intensity domains are present: M ($A_{UT} = 60$), H ($A_{UT} = 70$), VH ($A_{UT} = 80, 90, 100, 110$), S ($A_{UT} = 120$). The primary phase II of the $\dot{V}O_2$ on-kinetics and the magnitude of the slow component of the $\dot{V}O_2$ on-kinetics for particular exercise intensities are shown. The figure is truncated at 12 min for clarity.

Increasing ATP usage activity ($A_{UT}$, analogous to PO) affected significantly the $\dot{V}O_2$ on-kinetics. This is demonstrated in results from the default simulation of physically active muscle in Figure 2. The steady-state $\dot{V}O_2$ of the primary phase II of the muscle $\dot{V}O_2$ on-kinetics equals 7.1, 8.2, 9.4, 10.5, 11.7, 12.8 and 13.9 $mM \cdot min^{-1}$ for $A_{UT} = 60, 70, 80, 90, 100, 110$ and 120, respectively. $t_{0.63}$ changes little with work intensity (see [17] for discussion) and slightly increases from 24.2 s at $A_{UT} = 60$ to 25.6 s at $A_{UT} = 110$.

For $A_{UT} = 60$, muscle $\dot{V}O_2$ stabilizes at a steady-state soon after (2–3 min) the onset of exercise and the actual $\dot{V}O_2$ on-kinetics overlaps with the primary phase II of the $\dot{V}O_2$ on-kinetics. This is the M domain.

For $A_{UT} = 70$, the muscle $\dot{V}O_2$ on-kinetics first follows the exponential primary phase II kinetics, and then, after less than 2 min of exercise, the slow component of the muscle $\dot{V}O_2$ on-kinetics is activated due to $P_i$ reaching $Pi_{crit}$; this transition generates a characteristic 'notch' in the $\dot{V}O_2$ on-kinetics, at least for some ATP usage activities. However, afterwards, $\dot{V}O_2$ ultimately stabilizes at a greater $\dot{V}O_2$ than expected based on phase II $\dot{V}O_2$ kinetics, but below $\dot{V}O_{2max}$. A significant slow component can be observed. This simulation represents the H domain.

For $A_{UT} = 80, 90, 100$ and 110, muscle $\dot{V}O_2$ reaches $\dot{V}O_{2max}$ and the higher the $A_{UT}$, the sooner exercise is terminated. Here, the absolute magnitude of the slow component decreases with $A_{UT}$. The primary phase II of the $\dot{V}O_2$ on-kinetics does not project above $\dot{V}O_{2max}$. This is the VH domain.

Finally, for $A_{UT} = 120$, $\dot{V}O_2$ rapidly attains $\dot{V}O_{2max}$, at which exercise is terminated, the slow component is very small as it has very little time to develop and the actual $\dot{V}O_2$ on-kinetics is difficult to discern from the primary phase II of the $\dot{V}O_2$ on-kinetics. The primary phase II of the $\dot{V}O_2$ on-kinetics projects above $\dot{V}O_{2max}$. Therefore, this simulation represents S domain.

Muscle phosphate metabolite concentrations (ADP, PCr, Pi, $H_2PO_4^-$) and pH follow a similar pattern. This is shown in Figure 3. Metabolites also quickly reach a steady-state for $A_{UT} = 60$, reach a delayed and elevated steady state, with a 'notch' in their kinetics, for $A_{UT} = 70$ and absolute concentration changes are more rapid with increasing



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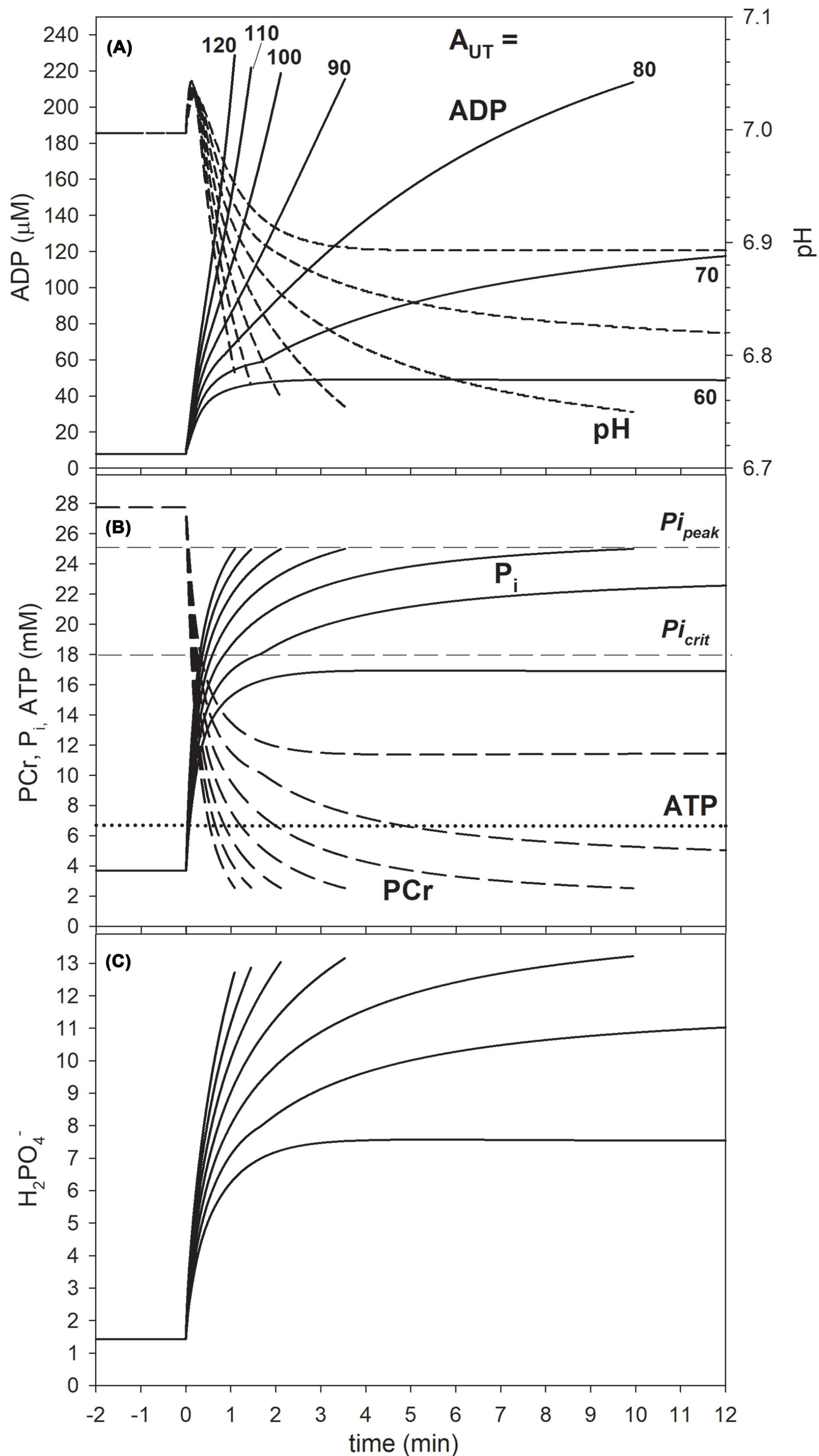


**Figure 3. Simulated on-kinetics of selected metabolites of the skeletal muscle bioenergetic system**
(**A**) ADP and pH; (**B**) PCr, $P_i$ and ATP; (**C**) $H_2PO4^-$.

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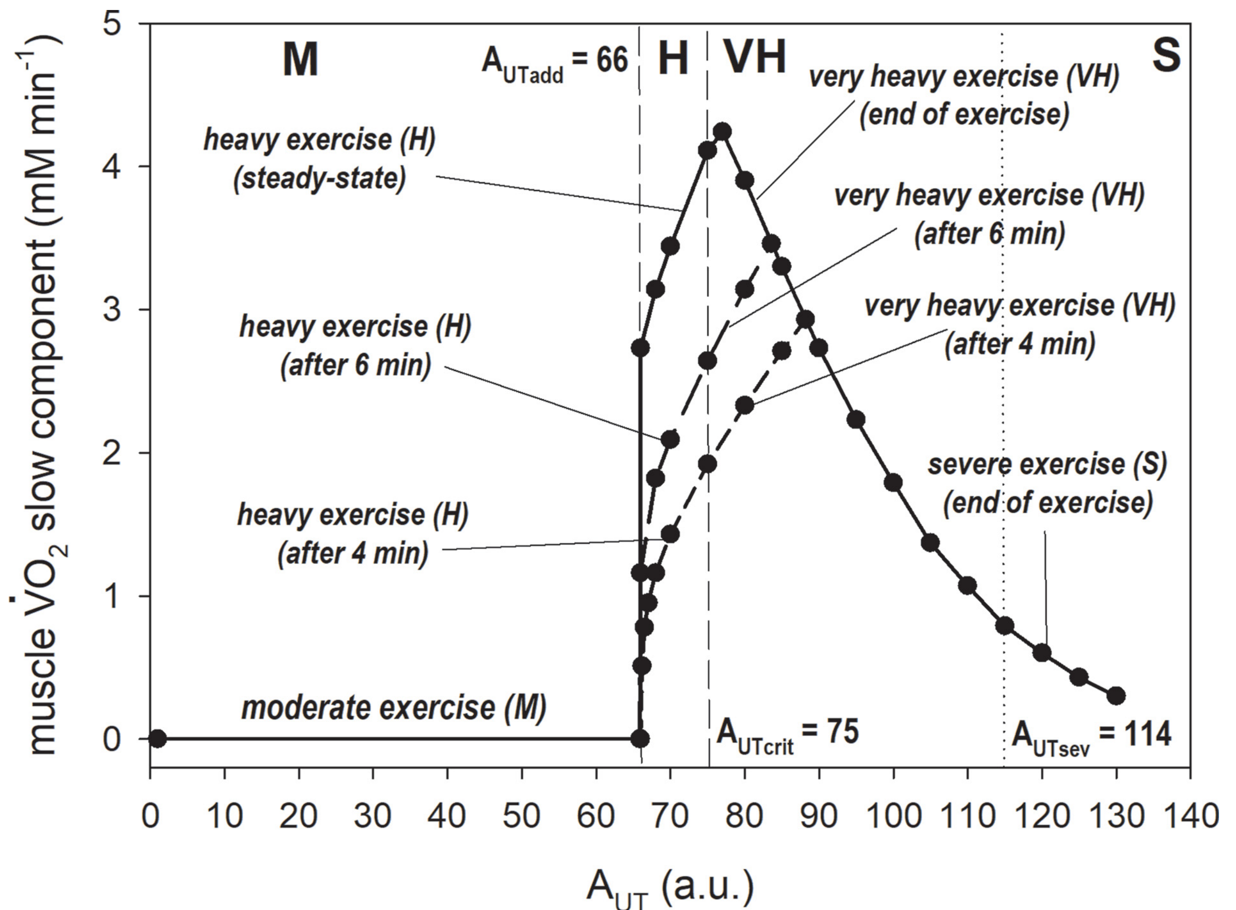


**Figure 4. Simulated dependence of the muscle $\dot{V}O_2$ slow component on ATP usage activity ($A_{UT}$, corresponding to PO)**
Slow component values at the termination of exercise are shown (upper curve; solid line). In addition, slow component values after 4 and 6 min of exercise are shown (lower two curves; dash line). Exercise intensity domains are separated by vertical dashed or dotted lines. The values of ATP usage activity ($A_{UT}$) at M/H border ($A_{UTadd}$), H/VH border ($A_{UTcrit}$) and VH/S border ($A_{UTsev}$) are indicated.

$A_{UT}$ values. For $A_{UT}$ values = 80 and above, end-exercise metabolite concentrations are identical for PCr and for $P_i$ (in the latter case by definition) and similar for ADP, $H^+$ and $H_2PO4^-$. The relative increase in $H_2PO4^-$ is larger (9.1 times for $A_{UT}$ = 100) than in $P_i$ (6.8 times), as the former is a derivative of both $P_i$ and $H^+$ increase.

The absolute value of the $\dot{V}O_2$ on-kinetics slow component, by definition absent in M domain, increases with $A_{UT}$ to maximal value immediately above the H/VH border. Then it decreases with $A_{UT}$ from the maximal value to low values in VH domain, and from low to very low values in the S domain. This is presented in Figure 4. In the S domain, the slow component has simply too little time to fully develop before termination of exercise because of fatigue.

The simulated values of $A_{UT}$ at the M/H border ($A_{UTadd}$), H/VH border ($A_{UTcrit}$) and VH/S border ($A_{UTsev}$) for normal, physically active muscle are presented in Figure 4 and in the middle row of Table 1. They equal $A_{UT}$ = 66, 75 and 114, respectively. The difference between $A_{UTadd}$ and $A_{UTcrit}$ (here, termed the 'H space') equals 9, while the difference between $A_{UTcrit}$ and $A_{UTsev}$ (or 'VH space') equals 39. In sedentary individuals (untrained muscle), the values of $A_{UTadd}$, $A_{UTcrit}$ and $A_{UTsev}$ are lower, than in normal muscle, H space is greater, while VH space is approximately the same (top row in Table 1). In endurance-trained muscle, $A_{UTadd}$, $A_{UTcrit}$ and $A_{UTsev}$ are greater than in normal physically active muscle, H space is reduced, while VH space is approximately the same (bottom row in Table 1). Thus, the training status is one of the factors determining the values of the M/H, H/VH and VH/S borders and the space between them.


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**Table 1 ATP usage activities at the borders between exercise intensity domains**

| Training status | ATP usage activity (a.u.) | | | | |
|---|---|---|---|---|---|
| | $A_{UTadd}$ | $A_{UTcrit}$ | $A_{UTsev}$ | H space | VH space |
| Untrained<br>$k_{OX}$ × 0.8, $Pi_{peak}$ = 27 mM | 52 | 68 | 107 | 16 | 39 |
| Physically active<br>$k_{OX}$ × 1.0, $Pi_{peak}$ = 25 mM | 66 | 75 | 114 | 9 | 39 |
| Endurance trained<br>$k_{OX}$ × 1.1, $Pi_{peak}$ = 24 mM | 73 | 79 | 116 | 6 | 37 |

The values of ATP usage activity at the border between the moderate (M) and heavy (H) exercise domain ($A_{UTadd}$), between the heavy and very heavy (VH) exercise domain ($A_{UTcrit}$) and between the very heavy and severe (S) exercise domain ($A_{UTsev}$). The space for heavy exercise: H space = $A_{UTcrit}$ - $A_{UTadd}$. The space for very heavy exercise: VH space = $A_{UTsev}$ - $A_{UTcrit}$.

# Discussion

## Biochemical origins of muscle exercise intensity domains

This study aimed to determine the origin of skeletal muscle exercise intensity domains at the biochemical/molecular level. In particular, our aim was to delineate these domains in terms of the '$P_i$ double-threshold' mechanism of muscle fatigue, comprising the $P_i$ on-kinetics, $Pi_{crit}$, $Pi_{peak}$ and the kinetics of the dependence of the additional ATP usage on the $P_i$-$Pi_{crit}$ difference.

We postulate that, in the M domain, the ATP usage activity ($A_{UT}$) is too small (below $A_{UTadd}$) for $P_i$ to exceed $Pi_{crit}$. Therefore, the additional ATP usage is not initiated and the system (fluxes and metabolite concentrations) quickly reaches a steady state. In the H domain, $A_{UT}$ is high enough (larger than $A_{UTadd}$) to cause $P_i$ to exceed $Pi_{crit}$ but too low (smaller than $A_{UTcrit}$) to bring $P_i$ (at a given additional ATP usage kinetics) to $Pi_{peak}$. Therefore, the additional ATP usage increases only temporarily, the slow component appears for a time, but ultimately the system stabilizes, albeit at a higher $\dot{V}O_2$ than that expected from the primary phase II kinetics (i.e., the additional ATP usage-$P_i$ positive feedback loop is too weak to cause a continuous increase in the additional ATP usage). In the H domain, $\dot{V}O_2$ does not reach $\dot{V}O_{2max}$ and exercise is not terminated because of fatigue (at least for the 30-min duration simulated here).

The value of $A_{UT}$ that is great enough to cause $P_i$ and additional ATP usage to progressively increase throughout exercise, where $P_i$ eventually reaches $Pi_{peak}$, is termed $A_{UTcrit}$. $A_{UTcrit}$ is an emerging feature of the bioenergetics system, and not a pre-determined value of $A_{UT}$ or $P_i$ (or other metabolite(s)). Below $A_{UTcrit}$ the positive feedback signal posed by mutual stimulation of $P_i$ increase and additional ATP usage increase is not strong enough for $P_i$ to reach $Pi_{peak}$ (and for $\dot{V}O_2$ to reach $\dot{V}O_{2max}$) and $\dot{V}O_2$ and $P_i$ and other metabolites can eventually stabilize. $A_{UTcrit}$ therefore is determined by the work rate (reflected in the absolute ATP usage activity) and the properties of the system itself, e.g. OXPHOS activity, ESA activity, $Pi_{peak}$, $Pi_{crit}$, $k_{add}$ (activity of the additional ATP usage) or $O_2$ concentration etc. [12,13]. When $A_{UT}$ exceeds $A_{UTcrit}$, the mutual stimulation (positive feedback) of $P_i$ increase and additional ATP usage increase is strong enough for $P_i$ to increase progressively throughout exercise and ultimately reach $Pi_{peak}$. At the same moment, $\dot{V}O_2$ reaches $\dot{V}O_{2max}$ and exercise is terminated because of fatigue. If $A_{UT} < A_{UTsev}$, the primary phase II of the $\dot{V}O_2$ on-kinetics does not project above $\dot{V}O_{2max}$ and the muscle is within the VH domain. If $A_{UT} > A_{UTsev}$, the primary phase II of the $\dot{V}O_2$ on-kinetics projects above $\dot{V}O_{2max}$ and the muscle enters the S domain.

Regarding the overall $\dot{V}O_2$ on-kinetics, there is no sharp border between VH and S intensity domains, as the VH domain passes smoothly (continuously) into the S domain. Rossiter [1] argued that in S intensity domain the slow component cannot be discerned from the primary phase II, rather than that it does not appear at all. The '$P_i$ double-threshold' approach supports this point of view, as the additional ATP usage, and thus the slow component, is always initiated once $P_i$ exceeds $Pi_{crit}$.

Thus, using our model, the muscle exercise domains may be characterized at the biochemical/molecular level in skeletal muscle fibers as follows and are detailed in Table 2:

- M domain – $P_i$ does not reach $Pi_{crit}$; no slow component is present; a steady-state is quickly reached; $\dot{V}O_2$ does not reach $\dot{V}O_{2max}$
- H domain – $P_i$ exceeds $Pi_{crit}$ but does not reach $Pi_{peak}$; a slow component is present, but a delayed steady-state is reached; $\dot{V}O_2$ does not reach $\dot{V}O_{2max}$

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**Table 2 Exercise intensity domains defined within the '$P_i$ double-threshold' mechanism of muscle fatigue**

| Property | Intensity domain | | | |
|---|---|---|---|---|
| | **Moderate (M)** | **Heavy (H)** | **Very heavy (VH)** | **Severe (S)** |
| $Pi_{crit}$ Exceeded | No | Yes | Yes | Yes |
| $Pi_{peak}$ Reached | No | No | Yes | Yes |
| Steady-state | Yes | Yes | No | No |
| Positive Feedback | No | Moderate | High | Very high |
| Slow Component | No | Moderate → High | High → Low | Low → Very low |
| Phase II $\dot{V}O_2$ exceeds $\dot{V}O_{2max}$ | No | No | No | Yes |

Particular domains are characterized by selected system properties.

- VH domain – $P_i$ exceeds $Pi_{crit}$ and ultimately reaches $Pi_{peak}$; $\dot{V}O_2$ reaches $\dot{V}O_{2max}$; exercise is terminated because of fatigue; the primary phase II of the $\dot{V}O_2$ on-kinetics does not exceed $\dot{V}O_{2max}$; the slow component of the $\dot{V}O_2$ (and metabolites) on-kinetics is required to bring $\dot{V}O_2$ to $\dot{V}O_{2max}$
- S domain – $P_i$ exceeds $Pi_{crit}$ and ultimately reaches $Pi_{peak}$; $\dot{V}O_2$ reaches $\dot{V}O_{2max}$; exercise is terminated because of fatigue; the slow component of the $\dot{V}O_2$ (and metabolites) on-kinetics has little time to develop, because the primary phase of the $\dot{V}O_2$ on-kinetics exceeds $\dot{V}O_{2max}$

It should be emphasized that the '$P_i$ double-threshold' mechanism used in this model is a deliberate simplification of the complex and numerous processes leading to muscle fatigue, reduced work efficiency and contributing to exercise intolerance. This is discussed in more detail in the "Study limitations" section.

It should also be clearly emphasized that $Pi_{crit}$ is directly related to $A_{UTadd}$, and not $A_{UTcrit}$ (analogous to CP). $Pi_{crit}$ is a parameter, while $A_{UTcrit}$ is an emergent property of the system, especially of the dependence of the additional ATP usage intensity on the $P_i$-$Pi_{crit}$ difference (involving the 'rate constant' of the additional ATP usage, $k_{add}$), $Pi_{crit}$ value and $Pi_{peak}$ value (see [12,13]). $A_{UTsev}$ is clearly related to $Pi_{peak}$ (affecting $\dot{V}O_{2max}$). $A_{UTadd}$, $A_{UTcrit}$ and $A_{UTsev}$ are also co-determined by the OXPHOS activity and ESA intensity, as they affect changes in $P_i$ during exercise [12,13].

## Muscle exercise intensity domains versus whole-body $\dot{V}O_2$ and blood $L^-$ kinetics

Of course, it is expected that exercise intensity domains at the muscle level underlie those at the whole-body level. However, a question arises whether the borders between the domains at both levels strictly overlap.

Using this model, the muscle and whole-body intensity domains can be related to each other through a conversion factor: one $A_{UT}$ unit of muscle ATP usage intensity is equivalent to about 3 W (2–4 W depending e.g. on working muscles mass) of the whole-body power output during cycling. This allows a relative scaling to be established between e.g. muscle $A_{UTcrit}$ and whole body CP, or % of muscle maximal $A_{UT}$ and whole-body $PO_{max}$ in ramp-incremental exercise. Alternatively, $A_{UT}$ can be described by muscle PO per unit muscle mass expressed in watt/kg.

It is not obvious that the skeletal muscle exercise intensity domains, determined mostly at the biochemical and molecular level, and whole-body exercise intensity domains at the physiological level, determined mostly on the basis of the pulmonary $\dot{V}O_2$ on-kinetics and blood $L^-$ (and $CO_2$) on-kinetics, should precisely overlap in each case. For instance, the whole-body M/H border determined by the $\dot{V}O_2$ on-kinetics and LT/GET could potentially differ from the muscle M/H border, which depends on $P_i$ exceeding $Pi_{crit}$. In particular, there seems to be no necessary reason that the fraction of the pulmonary slow component, originating predominantly in working muscles and in other tissues, should appear at the same time and PO/$A_{UT}$. The M/H border is defined at the whole body level by the emergence of the pulmonary $\dot{V}O_2$ slow component and/or the failure of blood $L^-$ to stabilize at (or close to) resting values and is analogous to LT/GET. On the other hand, M/H boarder in the muscle is defined by the highest $A_{UT}$ that does not cause $P_i$ to exceed $Pi_{crit}$. LT cannot be defined at the molecular/cellular level (single muscle fiber level) in the same way as it is at the whole-body (blood) level because blood lactate concentration during exercise is a result of the balance between lactate release by working muscle fibers and lactate uptake by non-working fibers and other tissues. Cytosolic lactate is a derivate of the rate of lactate/$H^+$ production by anaerobic glycolysis and the rate of lactate/$H^+$ efflux to blood. Consequently, some cytosolic acidification (noting also that muscle buffering capacity is less than the blood), and likely elevated cytosolic lactate concentration, is already present at POs/$A_{UT}$s that would be considered M exercise as defined at the muscle level by $P_i$ exceeding $Pi_{crit}$, or at the whole body level by pulmonary $\dot{V}O_2$ on-kinetics or



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blood $L^-$ measurements (see e.g., Cannon et al., 2014, where some muscle acidification appears in what is otherwise considered M exercise).

Poole et al. [18] showed that the contribution of working muscles to the whole body $\dot{V}O_2$ slow component is ~80% in VH exercise achieving $\dot{V}O_{2max}$ in approximately 20 min; other tissues such as cardiac, respiratory and accessory/stabilizing muscles are presumed to contribute the remaining ~20%. However, at lower POs in the H and VH domains, the fractional contribution of other tissues to the whole body $\dot{V}O_2$ slow component may be greater. Recognizing that other tissues contribute to the $\dot{V}O_2$ slow component, supports the idea that it is not necessary for the M/H border to occur at an identical PO/$A_{UT}$ at the whole body and muscle levels. Early muscle acidification and lactate accumulation, coupled with the contribution of other tissues to the pulmonary $\dot{V}O_2$ slow component, suggest that it is possible for the whole body M/H boarder to occur at a lower PO, and the $\dot{V}O_2$ slow component to start earlier in time, than their equivalents in the working muscle. Nevertheless, according to the present knowledge this is speculation. On the other hand, some dissociation of the pulmonary and muscle $\dot{V}O_2$ slow component kinetics can be seen in [19] (Figure 9A therein).

It is also possible that the M/H border determined from the pulmonary $\dot{V}O_2$ on-kinetics (lack or presence of the slow component) has a somewhat different value (in Watts or Watts per working muscle mass, for instance) than the M/H border determined from L- and $H^+$ increase in blood. There seems to be no causal relation between the onset of the (pulmonary) $\dot{V}O_2$ slow component, constituting, in fact, an 'excessive' oxygen uptake at a given work intensity and elevated lactate concentration in blood (despite the strong corellative association between these variables) [20]. In fact, an increase in the rate of anaerobic glycolytic ATP supply alone (that produces lactate), provided that all other variables are kept unchanged, would decrease oxidative ATP supply, and thus $\dot{V}O_2$ (the Crabtree effect), and not cause a disproportional increase (slow component). Also the elevated oxygen consumption by 'other tissues' is unlikely to be directly associated with elevated blood $L^-$, blood acidification or increased partial pressure of $CO_2$, becuase these tissues preferentially consume $L^-$ as a respiratory substrate. Finally, the $L^-$ concentration in arterial blood is a derivative of the balance between the $L^-$ release by active muscle and its uptake by other muscle and other tissues [21]. This balance does not have to be directly causally linked with the $\dot{V}O_2$ on-kinetics in active muscle or other tissues. Therefore, the similar values of the M/H border determined on the basis of the $\dot{V}O_2$ on-kinetics and from LT/GET seem likely to be an indirect association, related, but not directly causally linked with the event of $P_i$ exceeding $Pi_{crit}$.

On the other hand, in our opinion, the H/VH border is well- and uniquely-defined in terms of CP/$A_{UTcrit}$, both at the biochemical muscle and physiological whole-body level. It can be characterized as the highest PO/$A_{UT}$ at which $P_i$ and $\dot{V}O_2$ is able to stabilize and therefore $P_i$ does not reach $Pi_{peak}$ and $\dot{V}O_2$ does not reach $\dot{V}O_{2max}$. This behavior is a result of the intrinsic bioenergetic properties of the muscle. While the point of initiation (in time, PO or working muscle mass) of the $\dot{V}O_2$ and metabolite slow component can differ between the whole-body and biochemical muscle levels, it is the muscle bioergetic properties that determine muscle fatigue and the termination of exercise related to it (when $P_i$ reaches $Pi_{peak}$), both directly and through the action of the feedback to central nervous system [22–25].

In light of the ongoing debate about how to best characterize the highest PO at which whole-body physiologic variables stabilize, i.e. the H/VH border e.g. [26,27], it is worth noting that the H/VH borders determined on the basis of CP, maximal lactate steady-state (MLSS) or respiratory compensation point (RCP) do not have to overlap. The reason is that CP is a property of, and generated within, active muscles (see [14] for discussion), while MLSS and RCP are systemic events not only related to the muscle-generated CP but also influenced by other factors (the balance of lactate appearance and clearance, the senstitvity of the carotid body to an acidosis, or the absence of mechanical constraints limiting ventilation; [21,28,29]). We prefer to define the H/VH border on the basis of the active muscle-generated CP, as its intramuscular origin is unequivocal, is directly associated with muscle fatigue, and preceeds the later occruing systemic physiologic events.

The VH/S border can be defined, somewhat more abstractly, as PO/$A_{UT}$ at which $P_i$ would stabilize at (just below) the $Pi_{peak}$ value, and $\dot{V}O_2$ would stabilize just below $\dot{V}O_{2max}$, were the additional ATP usage, and thus the slow component of the $\dot{V}O_2$ on-kinetics, to be absent ('switched off').

## The magnitude of the slow component across exercise intensity domains

A dependence of the absolute value of the pulmonary $\dot{V}O_2$ slow component on PO was extracted in Poole and Jones [2] from experimental data presented in Poole et al. [30]. The simulated dependence of the muscle $\dot{V}O_2$ slow component on $A_{UT}$, shown here in Figure 4, is quite similar. The main difference between the computation and experimental data is that the simulation results in a narrower H space and a faster increase in slow component magnitude just above the

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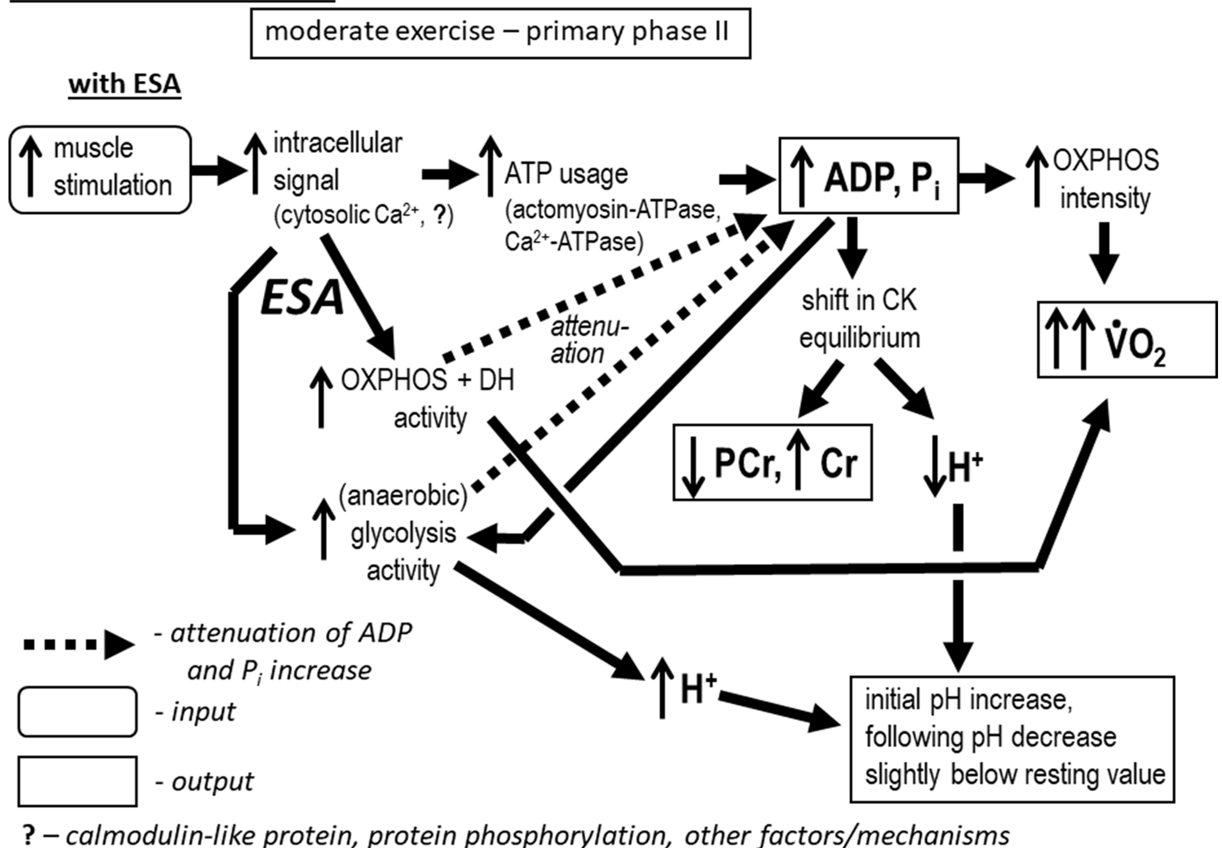


**Figure 5. Biochemical background of the primary phase II of the $\dot{V}O_2$ and metabolites on-kinetics**

Sequence of biochemical/molecular events (causal chain) in the bioenergetic system during rest-to-work transitions in skeletal muscle below the M/H border (M exercise intensity domain) in the presence of ESA. A detailed description is provided in the text.

M/H border. However, the experimental data contain only one point for the H domain, concealing a more detailed comparison.

At the muscle level in Figure 4, the H/VH border represents ~63–68% of the $A_{UTsev}$, which corresponds well to values of CP from whole body exercise that average 70% of the PO at $\dot{V}O_2$max (range: 53–80%, [5,31]). However, Figure 4 shows that the H/VH border is relatively low in the H+VH space range; it is ~15–30% of the $A_{UT}$ range between the M/H boarder and the VH/S border (depending on the training status of the muscle). This range, termed the 'delta' (%Δ) range in whole body studies e.g., Whipp (1996), is lower than expected based on CP from whole body exercise, which varies between ~15 and 60%Δ in healthy subjects [5,31]. This again supports the notion that the M/H boarder in whole body exercise may occur at a lower PO/$A_{UT}$ than its muscle equivalent, allowing the %Δ at which CP occurs to be greater at whole body compared with the muscle level. In addition, the VH/S boarder may also be lower in whole body exercise than at the muscular level, particularly in the trained state where muscle OXPHOS capacity (at least) can exceed the capacity for whole body $O_2$ delivery [32,33]. Therefore, both the M/H and VH/S borders may be significantly lower in whole body exercise, and thus the H space significantly broader in whole-body exercise compared with the isolated muscle.

## $\dot{V}O_2$ on-kinetics generation in particular exercise intensity domains

The $\dot{V}O_2$ on-kinetics in particular exercise intensity domains is an emergent property (epiphenomenon) of the biochemical bioenergetic system of skeletal muscle. Figures 5 and 6 describe the causal chain (sequence of events) from the input (muscle stimulation) to the outputs (chiefly $\dot{V}O_2$) in the primary phase II (Figure 5) and slow component (Figure 6) of the system on-kinetics. In this chain, preceding factors (before arrows: e.g. enzyme/metabolic block activities and metabolites) influence following factors (after arrows: enzyme/metabolic blocks activities, fluxes and metabolite concentrations) but not inversely. $\dot{V}O_2$ is a consequence of this chain of events and as such is not a causal



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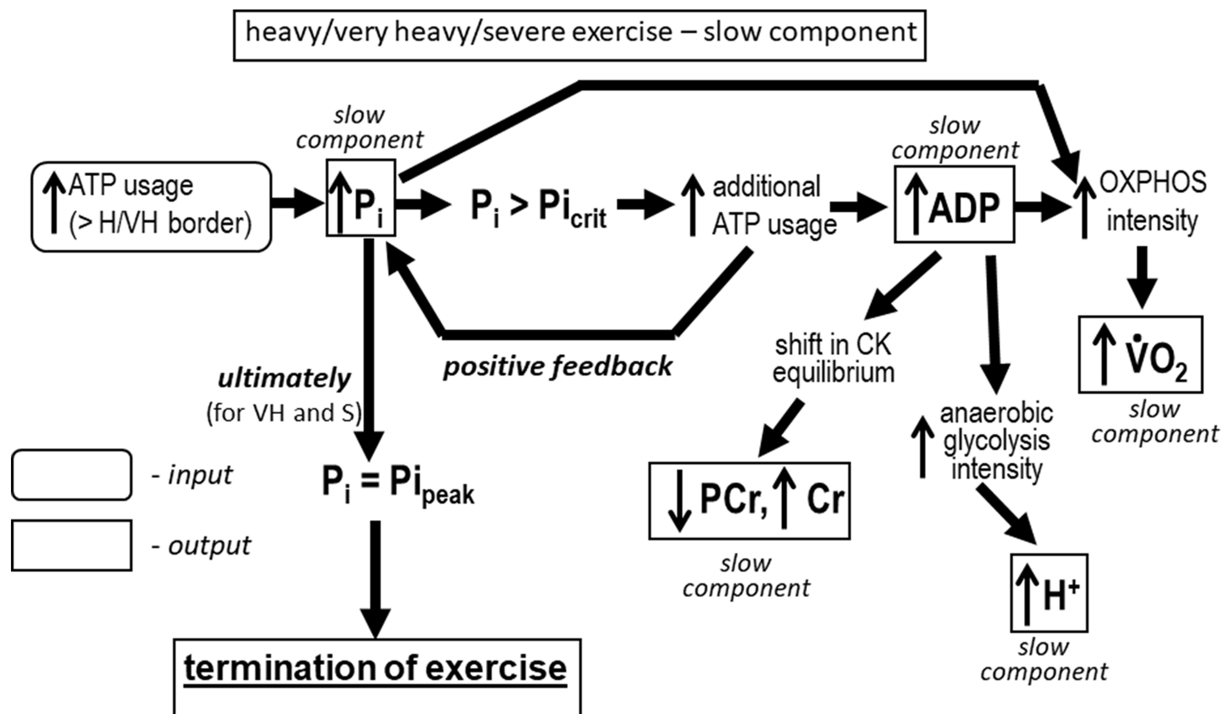


**Figure 6. Biochemical background of the slow component of the $\dot{V}O_2$ and metabolites on-kinetics**

Sequence of biochemical/molecular events (causal chain) in the bioenergetic system during rest-to-work transitions in skeletal muscle during exercise above the M/H boarder (H, VH and S exercise intensity domains) in addition to the system behavior below the M/H border depicted in Figure 5 (primary phase II of the $\dot{V}O_2$ and metabolites on-kinetics). A detailed description is provided in the text.

factor of system function; rather it is an epiphenomenon that may be used to non-invasively identify biochemical events originating in the muscle.

The sequence of biochemical/molecular events (causal chain) in the bioenergetic system during rest-to-work transition in skeletal muscle during moderate (M) exercise (primary phase II of the $\dot{V}O_2$ and metabolites on-kinetics) in the presence of each-step activation (ESA) is presented in Figure 5. Neural myocyte stimulation causes a release of $Ca^{2+}$ ions from sarcoplasmic reticulum. This in turn activates actomyosin-ATPase (muscle contraction) and $Ca^{2+}$-ATPase (SERCA). ATP is hydrolyzed to ADP and $P_i$, which elevates the concentration of the two latter (ATP concentration remains approximately constant because of the high ATP/ADP ratio, unless AMP deamination leads to a decrease of the total pool of adenine nucleotides). In parallel, essentially all elements of the system (perhaps with exception of the very fast CK), both cytosolic and mitochondrial, are directly activated by some factor/mechanism, probably related to $Ca^{2+}$, but likely involving also some other elements, e.g., calmodulin-like protein(s), 'presenting' $Ca^{2+}$ ions to enzymes/carriers and/or protein phosphorylation. This attenuates the increase in ADP and $P_i$ (in relation to the situation without ESA, [34], as lower accumulation of these metabolites is necessary in order for oxidative (and glycolytic) ATP supply to match the elevated ATP usage, because OXPHOS (and glycolysis) is already partly activated by ESA. The moderate ADP increase shifts the equilibrium of creatine kinase (CK), which leads to a moderate PCr decrease, Cr increase, consumption of $H^+$ (initial pH increase) and further moderate $P_i$ increase (concomitant action of CK and ATP usage). The moderate increase in ADP and $P_i$ stimulates OXPHOS, which leads to a significant increase in $\dot{V}O_2$ (OXPHOS is activated directly, and in parallel, through ESA). Because of moderate changes in metabolite concentrations, especially PCr, Cr and $P_i$, the transition time of the primary phase II of the $\dot{V}O_2$ and metabolites on kinetics ($t_{0.63}$ or $\tau_p$) is relatively short. ADP (and AMP) increase further activates (anaerobic) glycolysis. Production of $H^+$ by anaerobic glycolysis can slightly decrease pH below the resting value (e.g. [35]). However, accumulating protons inhibit (anaerobic) glycolysis, which prevents further significant cytosol acidification. Ultimately, the system reaches a steady-state.

Exercise in the H, VH and S domains entail an additional sequence of biochemical/molecular events (causal chain) in the muscle bioenergetic system supplementing the primary phase II of the system on-kinetics. This sequence of

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events underlies the slow components of the $\dot{V}O_2$ and metabolite on-kinetics (Figure 6). A work intensity (ATP usage activity) that is sufficiently high to cause $P_i$ to exceed critical $P_i$ ($Pi_{crit}$) initiates additional ATP usage (above that expected based on phase II kinetics). This, in turn, leads to a further increase in ADP and $P_i$, the latter further stimulating additional ATP usage, thereby forming a self-driving process (positive feedback loop). The increased ADP shifts the CK equilibrium, leading to a further decrease in PCr and increase in Cr. ADP (and AMP) further stimulates anaerobic glycolysis, which causes greater cytosol acidification. This in turn recursively inhibits (anaerobic) glycolysis (self-limiting process). The continuously increasing ADP and $P_i$ stimulate OXPHOS and thus lead to a further increase in $\dot{V}O_2$. As a result, the slow component in the $\dot{V}O_2$, $P_i$, PCr, Cr and $H^+$ on-kinetics appears. In H exercise, the mutual stimulation of the increase in $P_i$ and increase in the additional ATP usage is not strong enough for $P_i$ to reach $Pi_{peak}$ and thus for $\dot{V}O_2$ to reach $\dot{V}O_{2max}$. As a result, the system ultimately stabilizes, albeit at a higher steady-state than that expected in the absence of the additional ATP usage. The H/VH border is an emerging property of the system that separates POs/$A_{UT}$s for which this feedback loop can stabilize from those for which it cannot, i.e. $A_{UTcrit}$ in the muscle and CP at the whole body level. In VH and S exercise the mutual stimulation of the increase in $P_i$ and increase in the additional ATP usage is strong enough to prevent a steady-state from being achieved, $\dot{V}O_2$ increases and metabolites change continuously throughout exercise. Ultimately, $P_i$ reaches $Pi_{peak}$, $\dot{V}O_2$ reaches $\dot{V}O_{2max}$ and the exercise is terminated because of fatigue.

Work performed above CP (W' parameter of the power-duration dependence) has historically been termed 'anaerobic work capacity' or AWC. However, it should be emphasized that, under the conditions presented here for healthy individuals, the vast majority of ATP supply during exercise above CP ($A_{UTcrit}$) is by OXPHOS. Creatine kinase (CK) is the main ATP supplier in the initial seconds of exercise (first 20–30 s) [12,17], but this is also true for power outputs below CP ($A_{UTcrit}$).

Goulding et al. [36] collected numerous whole body experimental data demonstrating a close association between the $\dot{V}O_2$ on-kinetics ($t_{0.63}$ and/or $O_2$ deficit) and various system properties, especially CP. The simulations presented here emphasize that these associations from experimental data are not determined by $\dot{V}O_2$ on-kinetics, and that both CP and $\dot{V}O_2$ kinetics are emergent properties of the bioenergetic system. In the data presented by Goulding et al. [36], the $\dot{V}O_2$ on-kinetics represents a non-invasive characteristic (or proxy) that results from system parameters and variables, such as OXPHOS activity, ESA activity, $O_2$ concentration, or $Pi_{peak}$ [13]. The observed association between the $\dot{V}O_2$ on-kinetics and CP is consistent with computer simulations in that both these outputs result from parameters and variables of the system [13]. The inverse (negative) correlation between $t_{0.63}$ and CP observed in experimental studies results from the fact that the mentioned parameters change $t_{0.63}$ and CP in the opposite directions—compare e.g. two upper rows in Table 1 in [13]. A change in, e.g., total phosphate and/or creatine pool would work in a similar way. A similar reasoning can be applied to the $\dot{V}O_{2sc}$ ($\dot{V}O_2$ slow component) − W' (curvature constant of the power–duration relationship) relationship. Again, these emergent system properties are determined by several parameters, for instance $k_{add}$ - the 'rate constant' of the additional ATP usage. It is emphasized that $\dot{V}O_2$ and $\dot{V}O_2$ kinetics are epiphenomena, located at the end of the causal chains shown in Figures 5 and 6.

## Off-transients versus exercise intensity domains

During muscle recovery (off-transient) $P_i$ quickly falls below $Pi_{crit}$ (after ∼10–20 s, see e.g. Figure 5 in [17]). Therefore, according to the '$P_i$ double-threshold' mechanism, the additional ATP usage and thus slow component of the muscle $\dot{V}O_2$ off-kinetics quickly disappears. This conclusion conforms well to experimental observations concerning recovery after M and H exercise [37]. A slow approach of pulmonary $\dot{V}O_2$ to the resting value, resembling to some extent the slow component of the $\dot{V}O_2$ on-kinetics, was observed during recovery after VH exercise [37]. However, this phenomenon could be caused, at least partly, by a slow decay of ESA (during recovery OXPHOS produces ATP mostly for PCr resynthesis by CK), slowing the off-transient of the muscle (and therefore also pulmonary) $\dot{V}O_2$ off-kinetics [38]. Additionally, Krustrup et al. [19] demonstrated that pulmonary $\tau_p$ during off-transient is significantly longer, than muscle $\tau_p$. This can be due to a slow recovery of cardiac and respiratory muscle activity (heart rate and ventilation remain raised for many minutes following VH or S exercise) and/or $\dot{V}O_2$ in other tissues, re-filling of $O_2$ stores in tissues and blood, and circulatory distortion between muscle and pulmonary $\dot{V}O_2$. This conclusion is supported by the fact that such a 'slow component' of the $\dot{V}O_2$ off-kinetics is observed during recovery from intense exercise on the whole-body (pulmonary) level but not on the muscle level (see Figure 9B in [19]). In this case, the contribution of ESA decaying slowly, is likely low. Generally, the 'slow component' of the $\dot{V}O_2$ off-kinetics does not seem underlain by the additional ATP usage in working muscles, as is the slow component of the $\dot{V}O_2$ on-kinetics.



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## General discussion

In constant-power exercise of an isolated muscle group end-exercise PCr, pH and $P_i$ are similar for various work intensities [25]. In addition, when exercise tolerance is manipulated using alterations in oxygen delivery, end-exercise $P_i$, PCr and pH are similar [23]. These observations support the concept of $Pi_{peak}$. No thresholds are observed in biochemical studies concerning the relationship between $P_i$ and force generation in skinned fibers [39,40]. However, this system is very different from voluntary constant-power exercise in intact muscles, as it involves no cytosolic milieu, varying force, constant pH, constant external $Ca^{2+}$, no $Ca^{2+}$ handling and no ATP usage by $Ca^{2+}$-ATPase (SERCA). Unlike skinned fibers, task failure in isolated muscle constant-power exercise occurs when power production is no longer capable of meeting the task requirement, thus occurring at a common magnitude of peripheral fatigue [22,24]. Therefore, skinned fibers and intact muscle systems cannot be directly compared (see [14] for discussion).

The $P_i$ double-threshold mechanism can be sensibly defined in some types of exercise including voluntary constant-power exercise and perhaps ramp exercise and all-out exercise, although the values of $Pi_{peak}$ and $Pi_{crit}$ can be different in different exercise types. On the other hand, it is not clear whether this mechanism works in other cases, such as isometric exercise or electrically stimulated muscle.

## Study limitations

The dynamic model used for computer simulations in this study, as any model of this kind, constitutes only a simplification and approximation of the complex reality. For instance, it is a one-compartment model that does not distinguish different muscle fiber types and operates with parameters and variables (activities, fluxes, metabolite concentrations) averaged over the entire muscle. On the other hand, it is compared with 'one-compartment' experimental data: muscle (or pulmonary) $\dot{V}O_2$ and muscle PCr, $P_i$, ADP, ATP and $H^+$ concentrations. When doing this, the model is able to account, at least semi-quantitatively, for a surprisingly wide range of various kinetic properties of the skeletal muscle bioenergetic system.

The original '$P_i$ double-threshold' mechanism involves explicitly the total concentration of $P_i$ as the main peripheral-fatigue-related metabolite. However, it is possible that the deprotonated form of $P_i$ – $H_2PO_4^-$ is the factor that directly leads to muscle fatigue and exercise intolerance [41]. An advantage of this possibility is that the $H_2PO_4^-$ concentration is a derivative of $P_i$ and $H^+$ concentrations (acidification increases the fraction of $P_i$ being in the form of $H_2PO_4^-$), considered as two most important fatigue factors [9]. Additionally, the relative increase of $H_2PO_4^-$ during rest-to-work transition is greater than that of $P_i$ (9.1-fold versus 6.8-fold increase, see Figure 3). When $P_i$ is substituted by $H_2PO_4^-$ within the computer model, similar general theoretical results are obtained, although, of course, with different critical and peak values of $H_2PO_4^-$ (not shown).

The '$P_i$ double-threshold' mechanism is a deliberate simplification of the complex and numerous processes leading to muscle fatigue, reduced work efficiency and contributing to exercise intolerance. The precise quantitative details of these processes are yet to be determined, but most probably include action of other variables, especially $H^+$ and alteration in $Ca^{2+}$ release and sensitivity. On the other hand, as it is discussed in Korzeniewski [14], $P_i$ can cause $Ca^{2+}$ precipitation in sarcoplasmic reticulum and mediate in central fatigue (the central nervous system can sense somehow the metabolic state of working myocytes). For this reason, $P_i$ can be involved, directly or indirectly, also in $Ca^{2+}$-related and central fatigue, which might underlie the excellent agreement between computer simulations of the muscle cell and experimental data from intact working humans. Therefore, the $P_i$ double-threshold mechanism provides a useful working hypothesis, which produces quantitative features consistent with physiologic observation.

In addition, the extent to which whole-body $\dot{V}O_{2max}$ (as traditionally defined) is affected by systemic processes (such as convective and/or diffusive $O_2$ transport), rather than intramuscular limitations (potentially mediated by $P_i$, or neural feedback modulating motor activity), is dependent on the state of training [10,32]. In the skeletal muscle model used here, muscle $\dot{V}O_{2max}$ is effectively affected by $Pi_{peak}$, OXPHOS activity, ESA intensity and $O_2$, and the resultant behavior is consistent empirically with observations of $\dot{V}O_{2max}$ during whole-body exercise [12–14]. Nevertheless, increased limitations in $O_2$ supply and/or motor activation, or other non-muscle-molecular mechanisms, would be expected to reduce $A_{UTsev}$, lower the VH/S boarder and $\dot{V}O_{2max}$, and reduce the VH space, compared with the simulations presented here. It was shown [12] that a decrease in $O_2$ concentration decreases CP/$A_{UTcrit}$, and thus diminishes the H/VH border and H space.

Of course, at the present stage the $P_i$ double-threshold mechanism of muscle fatigue is only a hypothesis. Nevertheless, it can account for a surprisingly broad range of various, apparently unrelated, system properties [12–16]. In addition, recent experimental evidence appears broadly consistent with the $Pi_{crit}$ and $Pi_{peak}$ concepts [42]. Therefore, while this mechanism constitutes at best only a simplification and approximation of the reality, it contains properties that closely relate to experimental observations and therefore seem likely to contain at least some construct validity.

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Certainly, it will have to be ultimately verified or falsified by additional experimental studies. On the other hand, this concept has already stimulated and directed further experimental investigations [42]. The detailed molecular mechanism of the additional ATP usage underlying the slow component of $\dot{V}O_2$ and metabolites is not known (some possibilities are discussed in [14]) and will also have to be revealed in the experimental way. Nevertheless, the $P_i$ double-threshold mechanism can be regarded as a step towards a more detailed understanding of the phenomenon of muscle fatigue during constant power exercise.

Undoubtedly, the present model is still, to a significant extent, phenomenological, as it does not involve, e.g., the molecular mechanism driving additional ATP usage. Therefore, more detailed models will have to be developed constituting a refinement or extension of the present model.

# Conclusions

A detailed biochemical mechanism, through which the exercise intensity domains in skeletal muscle, namely moderate (M), heavy (H), very heavy (VH) and severe (S) intensity domains, originate at the biochemical/metabolic level of the myocyte is postulated. The genesis of exercise intensity domains and biochemical events in the skeletal muscle myocyte at the onset of exercise involves ESA regulation mechanism and is based on the '$P_i$ double-threshold' mechanism of muscle fatigue of a well-tested dynamic computer model of the skeletal muscle bioenergetic system developed previously. The '$P_i$ double-threshold' mechanism of muscle fatigue, a necessary simplification of complex system behaviors, is able to generate many various, apparently unrelated, system properties in sedentary, physically-active and endurance-trained muscle that reveal the muscular origins of the M/H, H/VH and VH/S exercise intensity boarders. Muscle training elevates the work intensities at which the M/H and H/VH borders appear and reduces the 'H space' i.e. the distance between these borders. It is argued that the value of PO (per working muscle mass) at the M/H border may be different (typically greater) at the skeletal muscle biochemical level compared with the whole-body physiological level. On the other hand, the PO at the H/VH border, above which a steady-state cannot be reached, seems identical in working skeletal muscle and whole-body exercise, and originates mostly in the former. Overall, this study demonstrates how characteristic physiologic responses to exercise over a wide range of intensities emerge, at least in part, from biochemical events at the level of the working skeletal muscle.

# Theoretical methods

## Computer model

The previously developed computer model of the skeletal muscle bioenergetic system, including detailed kinetic OXPHOS description, was used [12,17,43–46]. The model involves the ESA (parallel activation) mechanism, according to which ATP usage, NADH supply, glycolysis/glycogenolysis and all OXPHOS complexes are directly activated by some cytosolic factor/mechanism (likely to involve cytosolic $Ca^{2+}$ ions) during rest-to-work or low-to-high-work transitions in skeletal muscle, heart and other tissues [47,48]. A similar idea was proposed by Fell and Thomas in relation to other metabolic pathways, especially glycolysis [49,50]. The complete model description is given in [14] and located on the website: http://bernardkorzeniewski.pl.

A scheme of the skeletal muscle bioenergetic system is shown in Figure 1. The components of the system that are explicitly considered within the model are presented. The model comprises two main parts. The first is the set of kinetic equations that describe the dependence of the rate of particular enzymatic reactions, processes and metabolic blocks (NADH supply, glycolysis, ATP usage) on metabolite (substrate and product) concentrations. The second is the set of ordinary differential equations that describe the rates of change of particular metabolite concentrations in time: they equal the difference between the rates of all reactions/processes producing a given metabolite and the rates of all reactions/processes consuming it. These two parts form a recurrent, recursive loop: in each simulation time step new reaction/process rates are calculated on the basis of current metabolite concentrations, and new metabolite concentrations are calculated on the basis of current reaction/process rates.

This model was widely tested and was demonstrated to be able to reproduce a broad range of apparently unrelated kinetic properties of the skeletal muscle bioenergetic system, and was used for numerous theoretical studies [12–15,48].

## Computer simulations

Rate constants that appear in kinetic equations for all OXPHOS complexes (complex I, complex III, complex IV, ATP synthase, ATP/ADP carrier, $P_i$ carrier) and NADH supply block within the computer model ($k_{C1}$, $k_{C3}$, $k_{C4}$, $k_{SN}$, $k_{EX}$, $k_{PI}$, $k_{DH}$, respectively) can be grouped into a single rate constant of OXPHOS: $k_{OX}$, which corresponds to OXPHOS



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activity. In the standard model version, corresponding to normal, physically active individuals, the relative $k_{OX}$ is scaled to 1.

In this study three training states are considered, with three different values of $k_{OX}$ and $Pi_{peak}$ (compare [12,13]):

1. Normal, physically active individuals, $k_{OX} = 1.0$, $Pi_{peak} = 25$ mM
2. Untrained, sedentary individuals, $k_{OX} = 0.8$, $Pi_{peak} = 27$ mM
3. Endurance-trained individuals, $k_{OX} = 1.1$, $Pi_{peak} = 24$ mM

The time course of selected variable values (total muscle $\dot{V}O_2$, muscle $\dot{V}O_2$ of the primary phase II, cytosolic ADP, pH, ATP, PCr, $P_i$ and $H_2PO4^-$) during rest-to-work transition for increasing ATP usage activity (proportional to PO) ($A_{UT} = 60, 70, 80, 90, 100, 110, 120$, scaled to 1 at rest) were simulated. It should be noted that total $A_{UT}$ comprises resting $A_{UT} = 1$ (for basic processes sustaining the functioning of the cell) and unloaded-work-related $A_{UT} = 4$. The additional ATP usage (giving rise to the $\dot{V}O_2$ and metabolite slow components) is a function of the current $P_i$–$Pi_{crit}$ difference, and is initiated when $P_i$ exceeds the critical value ($Pi_{crit} = 18$ mM). Simulated exercise termination was defined as when $P_i$ reaches $Pi_{peak}$ [12]. Simulations comprised 30 min of exercise unless exercise was terminated sooner because of fatigue.

One $A_{UT}$ unit corresponds to approximately 3 W during whole body exercise (e.g. cycling). This value may vary (between about 2 and 4 W), depending on e.g. working muscle mass and type of exercise. Particular OXPHOS complexes, NADH supply block and glycolysis were activated with some delay in parallel with ATP usage at the onset of exercise through ESA (see e.g. [45]).

The values of the ATP usage activity ($A_{UT}$, analogous to PO) corresponding to the M/H border, H/VH border and VH/S border have been named $A_{UTadd}$ ($A_{UT}$ at which the additional ATP usage appears when $P_i$ reaches $Pi_{crit}$), $A_{UTcrit}$ (corresponding to CP, above which no steady-state in the system can be reached) and $A_{UTsev}$ (beginning of S domain), respectively.

## Data Availability

The complete model description is located on the web site: http://bernardkorzeniewski.pl and in the data base BioModels: MODEL2203310001.

## Competing Interests

Bernard Korzeniewski has no competing interests associated with the manuscript. Harry Rossiter declares support by grants from NIH (R01HL151452, R01HL153460, P50HD098593, R01DK122767, P2CHD086851) and the Tobacco Related Disease Research Program (T31IP1666). He reports consulting fees from Omniox Inc., and is involved in contracted clinical research with Boehringer Ingelheim, GlaxoSmithKline, Novartis, AstraZeneca, Astellas, United Therapeutics, Genentech and Regeneron. He is a visiting Professor at the University of Leeds, UK.

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## CRediT Author Contribution

**Bernard Korzeniewski:** Formal analysis, Supervision, Validation, Investigation, Visualization, Methodology, Writing—original draft, Writing—review & editing. **Harry B. Rossiter:** Validation, Investigation, Writing—original draft, Writing—review & editing.

## Ethics Approval

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

## Abbreviations

$A_{UT}$, ATP usage activity; $A_{UTadd}$, $A_{UT}$ at which the additional ATP usage is initiated, M/H border; $A_{UTcrit}$, critical $A_{UT}$, analogous to CP, H/VH border; $A_{UTsev}$, $A_{UT}$ avove which S appears, VH/Sborder; CK, creatine kinaseA; CP, critical power; ESA, each-step activation; GET, gas exchange threshold; H, heavy exercise intensity domain; H space, H/VH border - M/H border; LT, lactate threshold; M, moderate exercise intensity domain; MLSS, maximal lactate steady-state; OXPHOS, oxidative phosphorylation; $Pi_{crit}$, critical $P_i$, above which the additional ATP usage is initiated; $Pi_{peak}$, peak $P_i$, at which exercise is terminated because of fatigue; PO, power output; S, severe exercise intensity domain; $t_{0.63}$, time to reach 63% of the response amplitude (or the time-constant of an exponential); VH, very heavy exercise intensity domain; VH space, VH/S border - H/VH border; $\dot{V}O_2$, oxygen consumption; $\dot{V}O_{2max}$, maximal $\dot{V}O_2$.

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