Prior heavy exercise elevates pyruvate dehydrogenase activity and muscle oxygenation and speeds O₂ uptake kinetics during moderate exercise in older adults

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Gurd BJ, Peters SJ, Heigenhauser GJF, LeBlanc PJ, Doherty TJ, Paterson DH, Kowalchuk JM. Prior heavy exercise elevates pyruvate dehydrogenase activity and muscle oxygenation and speeds O₂ uptake kinetics during moderate exercise in older adults. Am J Physiol Regul Integr Comp Physiol 297: R877–R884, 2009. First published July 15, 2009; doi:10.1152/ajpregu.90848.2008.—The adaption of pulmonary oxygen uptake (V˙O₂p) kinetics during the transition to moderate-intensity exercise is slowed in older compared with younger adults; however, this response is faster following a prior bout of heavy-intensity exercise. We have examined V˙O₂p kinetics, pyruvate dehydrogenase (PDH) activation, muscle metabolite contents, and muscle deoxygenation in older adults [n = 6; 70 ± 5 (67–74) yr] during moderate-intensity exercise (Mod1) and during moderate-intensity exercise preceded by heavy-intensity warm-up exercise (Mod2). The phase 2 V˙O₂p time constant (τV˙O₂p) was reduced (P < 0.05) in Mod2 (29 ± 5 s) compared with Mod1 (39 ± 14 s), PDH activity was elevated (P < 0.05) at baseline prior to Mod1 (2.1 ± 0.6 vs. 1.2 ± 0.3 mmol acetyl-CoA·min⁻¹·kg wet wt⁻¹), and the delay in attaining end-exercise activity was abolished. Phosphocreatine breakdown during exercise was reduced (P < 0.05) at both 30 s and 6 min in Mod2 compared with Mod1. Near-infrared spectroscopy-derived indices of muscle oxygenation were elevated both prior to and throughout Mod2, while muscle deoxygenation kinetics were not different between exercise bouts consistent with elevated perfusion and O₂ availability. These results suggest that in older adults, faster V˙O₂p kinetics following prior heavy-intensity exercise are likely a result of prior activation of mitochondrial enzyme activity in combination with elevated muscle perfusion and O₂ availability.

V˙O₂ kinetics; ageing; pyruvate dehydrogenase; phosphocreatine
The purpose of this study was to examine the effect of a prior bout of heavy-intensity exercise on VO\textsubscript{2p} kinetics, PDH activation, muscle metabolite content, and muscle oxygenation in older adults during subsequent moderate-intensity exercise. We hypothesized that following a prior bout of heavy-intensity exercise, the transition to moderate-intensity exercise would be associated with 1) faster VO\textsubscript{2p} kinetics; 2) an elevated activity of PDH; 3) a decreased reliance on substrate-level phosphorylation, as indicated by a decreased breakdown of phosphocreatine (ΔPCr); and 4) elevated muscle oxygenation, as determined by near-infrared spectroscopy (NIRS).

METHODS

Subjects. Six older healthy male adults (age, 70 ± 5 yr; VO\textsubscript{2peak}, 31 ± 4 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}) volunteered and gave written informed consent to participate in the study. All subjects were recreationally active but not involved in a specific training program at the time of the study. The study was approved by The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects and conformed with the Declaration of Helsinki. Data from this group of older adults (4 out of 6) were used, in part, in a previous publication from our laboratory, which compared the responses during the transition to moderate-intensity exercise (without prior warm-up exercise) to the responses observed in a group of young adults (23).

Exercise protocol. Subjects reported to the laboratory on six separate occasions at approximately the same time of day and ~2 h after consuming a small meal high in carbohydrate and low in fat. On the first day of testing, subjects performed an incremental ramp exercise test (20 W/min) to the limit of tolerance on an electronically-braked cycle ergometer (model H-300-R; Lode) for determination of the estimated lactate threshold (θ\textsubscript{L}) and VO\textsubscript{2peak} (for definitions see Ref. 22). From the results of this ramp test, work rates (WR) were identified that elicited a steady-state VO\textsubscript{2p}, corresponding to ~90% θ\textsubscript{L} (i.e., moderate-intensity exercise), and Δ50% (VO\textsubscript{2p} at θ\textsubscript{L} plus ~50% of the difference between the VO\textsubscript{2p} at θ\textsubscript{L} and VO\textsubscript{2peak}; i.e., heavy-intensity exercise).

During four of the subsequent five visits to the laboratory, subjects performed two step transitions in WR of moderate intensity (Mod1 and Mod2) separated by a step increase in WR of heavy intensity, as previously described (22, 24, 51). Exercise was performed continuously; the duration of each step transition was 6 min, and each transition was preceded by 6 min of baseline cycling at 20 W. Changes in WR were initiated as a step function without warning to the subject. This continuous protocol was performed once each visit, resulting in four repetitions for each subject and condition. In one other visit, placed randomly among the final 2–5 visits, subjects repeated the protocol but with the cycling being interrupted and muscle biopsies being taken at 5 min of baseline (20 W) cycling and at 30 s and 360 s of the transition to each of Mod1 and Mod2 (see below).

VO\textsubscript{2p}, measurement. Gas exchange was measured using a low dead space (90 ml) bidirectional turbine (model VMM 110; Alpha Technologies) and mass spectrometry (AMIS 2000) as described previously (1, 11, 12, 22–24, 51).

NIRS. NIRS (model NIRO 300; Hamamatsu Photonics, Japan) was used to continuously measure changes in concentration of local muscle deoxy-(Δ[Hb\textsuperscript{a}]), oxy- (Δ[O\textsubscript{2}Hb]), and total (Δ[Hb\textsubscript{TOT}]) hemoglobin-mygoglobin of the vastus lateralis muscle of the right leg as mined by near-infrared spectroscopy (NIRS).

Calculations. Muscle content of free ADP (ADP\textsubscript{f}) was calculated by assuming equilibrium of the creatine kinase and adenyly kinase reactions (15). ADP\textsubscript{f} was calculated by using the measured ATP, creatine, PCr, estimated H\textsuperscript{+} concentration ([H\textsuperscript{+}\textsubscript{k}]), and the creatine kinase equilibrium constant of 1.66×10\textsuperscript{6}; [H\textsuperscript{+}\textsubscript{k}] was estimated from the measured muscle pyruvate and lactate contents as described by Sahlin et al. (50). Free inorganic phosphate (P\textsubscript{i}) was calculated by adding the estimated free phosphate content of 10.8 mmol·(kg dry wt)\textsuperscript{-1} (15) to the difference in PCr content relative to the baseline value.

Statistical analysis. Parameter estimates for VO\textsubscript{2p} and for the two moderate-intensity exercise bouts were compared using a one-way ANOVA for repeated measures with τ\textsubscript{VO\textsubscript{2p}} being compared using a one-tailed test. The active form of PDH and muscle metabolite contents were compared using a two-way ANOVA for repeated measures with main effects of exercise bout and time. Significant main effects and interactions were subsequently analyzed by using a Tukey’s post hoc test. The relationship between the Mod1, τ\textsubscript{VO\textsubscript{2p}} and the Mod1–Mod2, τ\textsubscript{VO\textsubscript{2p}} difference was determined by Pearson product moment correlation analysis. Statistical significance was accepted at P < 0.05. Data are presented as means ± SD.
RESULTS

VO₂p kinetics. In the present study, subjects exercised at a moderate-intensity of 88 ± 7 %θL [45% ± 6 VO₂peak; power output, 66 ± 14 W] and at a heavy-intensity of Δ50% [~80% VO₂peak; power output, 140 ± 28 W]. The VO₂p response profile during the transition to each bout of moderate-intensity exercise for a representative subject (including the exponential model best-fit line) is shown in Fig. 1, while the summary of the parameter estimates for the on-transient VO₂p response to Mod1, and Mod2 is presented in Table 1. The phase 2 VO₂p time constant (τVO₂p) was reduced (P < 0.05) in Mod2 (29 ± 5 s) compared with Mod1 (39 ± 14 s). A significant correlation (r = 0.904, P < 0.05) existed between the time course of adaptation of VO₂p during Mod1 (τVO₂p Mod1) and the reduction in τVO₂p that occurred during Mod2 [ΔτVO₂p (Mod1 − Mod2)].

Muscle PDH activity. During Mod1, PDH activity was increased above baseline (1.15 ± 0.29 mmol acetyl-CoA·min⁻¹·kg wt⁻¹) at 30-s exercise (1.69 ± 0.67 mmol acetyl-CoA·min⁻¹·kg wt⁻¹) with a further increase (P < 0.05) observed at 6-min exercise (2.31 ± 0.42 mmol acetyl-CoA·min⁻¹·kg wt⁻¹). After heavy-intensity exercise, PDH activity was elevated (P < 0.05) at baseline (2.13 ± 0.63 mmol acetyl-CoA·min⁻¹·kg wt⁻¹), with no changes observed at 30-s exercise (2.05 ± 0.58 mmol acetyl-CoA·min⁻¹·kg wt⁻¹) or 6-min exercise in Mod2 (2.55 ± 0.74 mmol acetyl-CoA·min⁻¹·kg wt⁻¹). PDH activity was not different between Mod1 and Mod2 at 30 s and 6 min of the exercise transition (Fig. 2). The apparent trend between the change in τVO₂p and the change in PDH activity from Mod1 to Mod2 at baseline (Fig. 3A; P = 0.18) and 30 s of exercise (Fig. 3B; P = 0.20) did not achieve statistical significance.

Muscle metabolite content. Muscle pyruvate, lactate, and acetyl-CoA contents, and calculated [H⁺] are presented in Table 2. Muscle pyruvate content did not change significantly during exercise or as a consequence of prior heavy-intensity exercise. Muscle lactate content and [H⁺], were greater in Mod2 than Mod1 at baseline and after 30-s exercise. Acetyl-CoA content remained at baseline levels throughout Mod1. After heavy-intensity exercise, acetyl-CoA was greater (P < 0.05) at all times compared with Mod1 (Table 2).

Muscle contents of creatine, PCr, ATP, calculated ADPf, and P, are presented in Table 3. Absolute PCr content and PCr breakdown (ΔPCr) are presented in Fig. 4, A and B, respectively. During Mod1, PCr content tended to be lower (P = 0.06) at 30-s exercise (64.8 ± 14.5 mmol·kg dry wt⁻¹) compared with baseline (83.7 ± 4.9 mmol·kg dry wt⁻¹), and was significantly lower (P < 0.05) after 6-min exercise (51.7 ± 17.9 mmol·kg dry wt⁻¹). Baseline PCr content was lower (P < 0.05) prior to Mod2 than Mod1. During the transition to Mod2 a significant decrease (P < 0.05) in PCr content was not observed until 6-min exercise (Table 3); PCr breakdown from baseline (ΔPCr) was reduced (P < 0.05) at both 30 s and 6 min in Mod2 compared with Mod1 (Fig. 4B). Changes in muscle Cr content were similar but opposite to those observed for PCr content.

Muscle ATP content was unchanged compared with baseline during both Mod1 and Mod2 (Table 3). Muscle ADPf content increased (P < 0.05) at both 30-s and 6-min exercise in Mod1. Although muscle ADPf content tended to be higher at baseline in Mod2 compared with Mod1, these apparent differences were not significant. In Mod2 a significant increase (P < 0.05) in muscle ADPf content was seen only after 6-min exercise (Table 3); the increase in muscle ADPf content after 6-min exercise was ~60% lower in Mod2 than Mod1. Increases (P < 0.05) in muscle P content were seen after 6-min exercise in Mod1 and Mod2, with the increase in P content being ~45–60% lower in Mod2 than in Mod1 (Table 3).

NIRS-derived muscle oxygenation. Δ[HbTOT] decreased (P < 0.05) from baseline to end-exercise in both Mod1 and Mod2 and was elevated at both baseline and end-exercise in Mod2 compared with Mod1. Δ[HbTOT] increased (P < 0.05) from baseline to end-exercise in Mod1 and remained unchanged throughout Mod2; Δ[HbTOT] was greater (P < 0.05) at baseline

Fig. 1. Absolute pulmonary oxygen uptake (VO₂p) response of a representative subject (τMod1 = 63 s; τMod2 = 37 s) with line of best fit for Mod1 (black circle, grey line of best fit) and Mod2 (white circle, black line of best fit) with inset showing ΔVO₂p (0–100% end exercise) with line of best fit. Mod1, moderate-intensity exercise; Mod2, moderate-intensity exercise preceded by heavy-intensity warm-up exercise.
end-exercise in Mod2 compared with Mod1 (Table 4). There were no significant differences in the time course of the $/HHb$ response (i.e., $/HHb$-TD, $/HHb$-MRT) between Mod1 and Mod2 (Table 4), despite a significant reduction in the $/V_{O2p}$ in Mod2.

DISCUSSION

This is the first study to examine the adaptation of $V_{O2p}$ kinetics reflecting the kinetics of muscle $O_2$ utilization (2, 21, 38), activation of the mitochondrial PDH complex, changes in muscle metabolite concentrations, and changes in muscle oxygenation during the transition to moderate-intensity exercise before (Mod1) and after (Mod2) a priming bout of heavy-intensity exercise in older adults. The major new findings of the present study were that in older adults, the faster $V_{O2p}$ kinetics seen during the transition to Mod2 was coincident with a significantly higher preexercise PDH activity, which was not different from that seen in the steady-state of either Mod2 or Mod1, elevated NIRS-derived markers of local muscle $O_2$ availability (i.e., $\Delta[O_2Hb]$ and $\Delta[HbTOT]$), higher muscle contents of ADP$_r$ and Pi, and lower preexercise muscle PCr content. Thus after heavy-intensity priming exercise in older adults there was a greater availability of oxidative substrate prior to the onset of Mod2, which likely attenuated the delay required for increased mitochondrial delivery of oxidative substrates (reducing equivalents, ADP, Pi, and $O_2$ delivery) during the transition to exercise and resulted in faster activation of oxidative phosphorylation as implied by the faster $V_{O2p}$ kinetics during the onset of Mod2 compared with Mod1.

![Fig. 2](http://www.ajpregu.org)

Fig. 2. Pyruvate dehydrogenase activity of the active form (PDHa) at rest and at 30 s and 6 min of moderate-intensity exercise during Mod1 (black bars) and Mod2 (white bars). Values are mean ± SD *$P < 0.05$ vs. Mod1; †$P < 0.05$ vs. baseline; ‡$P < 0.05$ vs. 30 s.

![Fig. 3](http://www.ajpregu.org)

Fig. 3. Relationship between the change in PDHa (Mod2 to Mod1) and change in $V_{O2p}$ at baseline ($r = 0.63$, $P = 0.18$) and 30 s of exercise ($r = 0.61$, $P = 0.20$).

<table>
<thead>
<tr>
<th>Condition</th>
<th>Baseline</th>
<th>30 s</th>
<th>360 s</th>
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<tbody>
<tr>
<td><strong>Pyruvate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mod1</td>
<td>0.29 ± 0.16</td>
<td>0.35 ± 0.18</td>
<td>0.35 ± 0.25</td>
</tr>
<tr>
<td>Mod2</td>
<td>0.53 ± 0.25</td>
<td>0.39 ± 0.22</td>
<td>0.37 ± 0.21</td>
</tr>
<tr>
<td><strong>Lactate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mod1</td>
<td>10.5 ± 3.6</td>
<td>9.7 ± 1.2</td>
<td>18.6 ± 12.6</td>
</tr>
<tr>
<td>Mod2</td>
<td>28.5 ± 11.8*</td>
<td>25.6 ± 15.5*</td>
<td>23.1 ± 9.3*</td>
</tr>
<tr>
<td><strong>[H$^+$],10$^{-5}$ mol/l</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mod1</td>
<td>96.6 ± 3.2</td>
<td>95.8 ± 1.0</td>
<td>104.9 ± 12.7</td>
</tr>
<tr>
<td>Mod2</td>
<td>115.4 ± 12.9*</td>
<td>112.4 ± 16.3*</td>
<td>109.2 ± 9.4</td>
</tr>
<tr>
<td><strong>Acetyl-CoA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mod1</td>
<td>3.7 ± 1.7</td>
<td>4.0 ± 0.5</td>
<td>5.5 ± 1.0</td>
</tr>
<tr>
<td>Mod2</td>
<td>7.2 ± 1.2*</td>
<td>7.4 ± 1.9*</td>
<td>7.7 ± 1.2*</td>
</tr>
</tbody>
</table>

Values are means ± SD ($n = 6$ for pyruvate, lactate, and [H$^+$]; $n = 5$ for acetyl-CoA) in mmol/kg dry wt. *$P < 0.05$ vs. Mod1.
relationship exists between the previous findings (12, 51). Previously, we reported that a linear relationship was seen also in the present study (measurable reduction in phase 2 $V\dot{O}_2$ kinetics (bout of heavy-intensity exercise (i.e., $V\dot{O}_2$)). Consistent with the faster $V\dot{O}_2$ kinetics observed during that transition. Also, while muscle lactate content increased as a consequence of the heavy-intensity exercise, it reflects decreased lactate production through glycolysis, enhanced conversion to pyruvate (catalyzed by LDH), and subsequent oxidation through the mitochondrial PDH reaction, enhanced lactate efflux from muscle, or a combination of these factors during the transition to Mod2 (18).

Integrative control of oxidative phosphorylation in aging. We have argued previously (22, 24) that the kinetics of muscle $O_2$ utilization and oxidative phosphorylation, (as reflected by $V\dot{O}_2$) in young adults are not limited by a single substrate but rather by the combined availability of $O_2$, NADH (and FADH$_2$), ADP, and P$_i$ (56), a contention that recently has gained general acceptance (42). The findings of the current study demonstrating faster $V\dot{O}_2$ kinetics during Mod2 coincident with elevated PDH activity, elevated NIRS-derived markers of local muscle $O_2$ availability, greater muscle content of ADP and P$_i$, and lower PCr content prior to and early in exercise are consistent with $V\dot{O}_2$ kinetics in older adults being constrained by any one or the combined availability of these substrates in the unprimed Mod1 condition.

PDH activity, oxygenation, and muscle ADP concentration. The mitochondrial PDH complex catalyzes the rate-limiting step for provision of carbohydrate-derived substrate to the TCA cycle (in the form of acetyl-CoA) and ETC (reducing equivalents in the form of NADH) at the onset of exercise. However, the role of PDH activation in constraining the increase in oxidative metabolism, and therefore muscle $O_2$ utilization and $V\dot{O}_2$ kinetics, is controversial. Several studies have demonstrated a decrease in substrate-level phosphoryla-

![Fig. 4. Muscle phosphocreatine (PCr) at baseline and at 30 s and 6 min of moderate-intensity exercise during Mod1 (black bars) and Mod2 (white bars) (A) with net breakdown of PCr ($\Delta$PCr) in Mod1 (black bar) and Mod2 (white bar) between baseline and 30 s and baseline and 360 s (B). Values are means ± SD in mmol/kg dry wt. *P < 0.05 vs. Mod1; †P < 0.05 vs. baseline.](http://ajpregu.physiology.org/10.1152/ajpregu.00830.2008)

Table 3. Muscle contents of creatine, ATP, calculated free ADP (ADPf), and Pi during Mod1 and Mod2 in older adults

<table>
<thead>
<tr>
<th>Condition</th>
<th>Baseline</th>
<th>30 s</th>
<th>360 s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mod1</td>
<td>12.0±6.8</td>
<td>30.6±7.4†</td>
<td>44.9±9.9†</td>
</tr>
<tr>
<td>Mod2</td>
<td>21.1±7.5*</td>
<td>27.2±6.7</td>
<td>35.3±8.2†</td>
</tr>
<tr>
<td>PCr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mod1</td>
<td>83.7±4.9</td>
<td>64.8±14.5</td>
<td>51.7±17.9†</td>
</tr>
<tr>
<td>Mod2</td>
<td>75.8±7.1*</td>
<td>68.2±6.0</td>
<td>61.1±9.2†</td>
</tr>
<tr>
<td>ATP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mod1</td>
<td>32.8±10.5</td>
<td>30.7±10.6</td>
<td>29.9±9.2</td>
</tr>
<tr>
<td>Mod2</td>
<td>27.2±4.8</td>
<td>32.1±6.7</td>
<td>25.1±2.9</td>
</tr>
<tr>
<td>ADPf</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mod1</td>
<td>48.6±12.6</td>
<td>99.5±46.5†</td>
<td>149.8±34.2‡†</td>
</tr>
<tr>
<td>Mod2</td>
<td>66.5±34.4</td>
<td>90.7±35.0</td>
<td>106.4±53.2‡†</td>
</tr>
<tr>
<td>Pi</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mod1</td>
<td>10.8</td>
<td>29.4±17.2‡</td>
<td>41.7±24.4†</td>
</tr>
<tr>
<td>Mod2</td>
<td>19.9±9.5</td>
<td>27.1±9.4</td>
<td>34.1±13.1†</td>
</tr>
</tbody>
</table>

Values are means ± SD (n = 6) in mmol/kg dry wt except ADPf which is in μmol/kg dry wt. PCr: phosphocreatine. *P < 0.05 vs. Mod1; †P < 0.05 vs. baseline. ‡P < 0.05 vs. 30 s. 

$V\dot{O}_2$ kinetics. In the present study, the time constant for the phase 2 $V\dot{O}_2$ kinetics ($\tau V\dot{O}_2$) estimated during the transition to Mod1 in older adults (~39 s) was similar to that reported previously in older adults (~40–50 s), and was greater than that normally found in younger adults (~20–25 s) (1, 5, 9, 11, 12, 22–24, 51). In addition, in the present study, the $\tau V\dot{O}_2$ for older adults was reduced when moderate-intensity exercise was performed subsequent to a bout of heavy-intensity exercise ($\tau V\dot{O}_2$ Mod1, ~29 s; $\tau V\dot{O}_2$ Mod1, ~39 s), consistent with previous findings (12, 51). Previously, we reported that a linear relationship exists between the $\tau V\dot{O}_2$ estimated during the transition to Mod1 (i.e., without prior heavy-intensity exercise) and the reduction in the $\tau V\dot{O}_2$ that occurs subsequent to a prior bout of heavy-intensity exercise (i.e., $\tau V\dot{O}_2$ Mod1 – $\tau V\dot{O}_2$ Mod2) in both young (22, 24) and older adults (51). This relationship was seen also in the present study ($r = 0.904$) and supports the contention that the likelihood of observing a measurable reduction in $\tau V\dot{O}_2$ is greater in subjects presenting with slower (i.e., $\tau V\dot{O}_2$ ~40s) rather than faster (i.e., $\tau V\dot{O}_2$ ~20 s) $V\dot{O}_2$ kinetics.

Faster $V\dot{O}_2$ kinetics, reflecting faster activation of mitochondrial oxidative phosphorylation and muscle $O_2$ utilization (2, 21, 38) is expected to reduce the requirement for substrate-level phosphorylation (and nonoxidative ATP synthesis), thereby lowering PCr breakdown and lactate accumulation during the exercise transition (7, 31, 40, 46, 53). In the present study, PCr breakdown was reduced in Mod2 compared with Mod1 (Fig. 4), consistent with the faster $V\dot{O}_2$ kinetics observed during that transition. Also, while muscle lactate content increased as a consequence of the heavy-intensity exercise, muscle lactate content decreased during Mod2 (Table 2), reflecting decreased lactate production through glycolysis, enhanced conversion to pyruvate (catalyzed by LDH), and subsequent oxidation through the mitochondrial PDH reaction, enhanced lactate efflux from muscle, or a combination of these factors during the transition to Mod2 (18).
Table 4. Summary of parameter estimates for near-infrared spectroscopy response to cycle ergometer exercise in Mod1 and Mod2 in older adults

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mod1</th>
<th>Mod2</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\Delta [O_2Hb])</td>
<td>1.8±4.7</td>
<td>22.8±5.7*</td>
</tr>
<tr>
<td>End exercise, (\mu M)</td>
<td>-1.5±3.7†</td>
<td>6.0±3.2†</td>
</tr>
<tr>
<td>(\Delta [HHb])</td>
<td>-5.0±9.1</td>
<td>15.8±9.3*</td>
</tr>
<tr>
<td>End exercise, (\mu M)</td>
<td>4.4±8.8†</td>
<td>13.6±8.7*</td>
</tr>
</tbody>
</table>

Values are means ± SD (n = 6) and represent the change [Hb] from a 0 set baseline before the start of cycling exercise. \(\Delta [HHb]\)-TD, time delay; \(\Delta [HHb]\), \(\Delta [HHb]\) time constant; HHb-MRT. HHb mean response time (\(\tau + TD\)). *\(P < 0.05\) vs. Mod1; †\(P < 0.05\) vs. baseline.

In contrast to young adults in this and in our previous study (23), there was a demonstrated delay in achieving the full, steady-state activation of PDH in older adults. These results are possibly consistent with an attenuated activation of PDH contributing, in part, to the relatively slow VO\(_2\)p kinetics observed in older adults in Mod1, while the elimination of this delay during the transition to Mod2, a consequence of prior exercise, would be expected to deliver greater substrate to the TCA cycle and thus possibly contribute to the faster VO\(_2\)p kinetics in Mod2. In this study, a significant relationship between the changes in \(\tau\)VO\(_2\) and the changes in PDH activity was not observed between Mod1 and Mod2 at either baseline (\(P = 0.18\); Fig. 3A) or 30 s of exercise (\(P = 0.20\); Fig. 3B), despite a reasonably strong positive relationship being seen in this limited number of older subjects (\(r = 0.6\)). However, we cannot dismiss the findings that in older adults having slowed PDH activation kinetics and slow VO\(_2\)p kinetics, a prior bout of heavy-intensity exercise resulted in faster VO\(_2\)p kinetics and that this speeding occurred in association with an elevated PDH activity and removal of the delay in activating PDH to the required steady-state level.

PDH activation can also provide information about the activation status of other rate-determining enzymes, specifically within the TCA cycle, as these enzymes are activated by many of the same metabolites responsible for activating PDH (52). Thus the elevated PDH activity prior to Mod2 suggests that other dehydrogenases within the TCA cycle also were activated to increase TCA cycle flux and provide reducing equivalents for the ETC during the transition to Mod2. In support of this is the observation that contraction-induced activation of oxidative phosphorylation in Xenopus single muscle fibers resulted in a faster fall in intracellular O\(_2\) gauge recorded by a DCA-mediated activation of PDH (estimated MRT \(~\sim\)33 s (32)), suggesting that full activation of oxidative phosphorylation requires more than just priming the mitochondrial PDH complex.

In addition to elevated PDH activity, the NIRS-derived concentration changes in muscle oxy- \(\Delta [O_2Hb]\) and total \(\Delta [HHb]\) hemoglobin-myoglobin were both elevated following the heavy-intensity exercise and throughout Mod2. These findings are consistent with previous studies examining prior exercise in both young and older adults and suggest a greater muscle perfusion prior to and throughout Mod2 (12, 22, 24). A greater muscle oxygenation is consistent with previous demonstrations of elevated HR in older adults (12, 51) and greater limb conduit artery blood flow in young adults (16, 17, 41) during baseline exercise following heavy-intensity exercise and very early in the transition to a subsequent bout of exercise. There is evidence that O\(_2\) availability is impaired during the transition to exercise in muscle from older animals and human subjects (3, 11, 12, 14, 39, 43, 44, 49), and thus prior exercise in older adults likely improves O\(_2\) availability in Mod2 compared with Mod1. A greater muscle perfusion would improve the ratio of local muscle perfusion to O\(_2\) utilization during the exercise transition, and thereby reduce the reliance on fractional O\(_2\) extraction. Given an increase in perfusion (relative to O\(_2\) utilization), the fall in microvascular PO\(_2\) should be attenuated, thereby maintaining a higher O\(_2\) driving pressure for diffusive O\(_2\) delivery from the microvascular red blood cell to the muscle mitochondria. Although Behnke et al. (4) reported a more rapid fall in microvascular PO\(_2\) during the second of two contraction bouts in rat spinotrapezius muscle preparations, likely a consequence of faster muscle O\(_2\) utilization kinetics, a transient undershoot in microvascular PO\(_2\), which is a feature of older animal preparations (3) was not observed, suggesting the limitations may be different in young and older animals. Also, the greater \(\Delta [HHb]\) (and microvascular hematocrit) would increase the functional capillary surface area for O\(_2\) diffusion, thereby contributing to a higher diffusional O\(_2\) conductance between the microvasculature and muscle.

Changes in the NIRS-derived deoxyhemoglobin concentration (\(\Delta [HHb]\)) reflect the balance between O\(_2\) availability and utilization in the microvasculature surrounding the muscle and therefore provide an index of fractional O\(_2\) extraction. In the present study, there was no significant difference in the time course of \(\Delta [HHb]\), although the group mean \(\tau\Delta [HHb]\) and \(\Delta [HHb]\)-MRT were both \(~\sim\)13 s greater in Mod2 despite faster VO\(_2\)p kinetics (\(\tau\)VO\(_2\)p \(~\sim\)10 s lower in Mod2). The similar (or trend towards slower) deoxygenation kinetics agrees with previous reports (12, 22) and is consistent with a greater muscle blood flow and O\(_2\) delivery to muscle O\(_2\) demand during the transition to Mod2.

A higher mitochondrial ADP\(_f\) and P\(_i\) at baseline also may have contributed to the faster VO\(_2\)p kinetics observed in this study during Mod2. Baseline muscle PCR content was lower in Mod2 compared with Mod1 likely due to inadequate recovery time. This is consistent with findings of lower baseline PCR following heavy-intensity exercise from both biopsy studies (18) and magnetic resonance spectroscopy (48). It was dem-

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onstrated recently that an intact PCr system serves as an energy buffer to limit the increase in cytosolic and mitochondrial ADP. In studies utilizing iodoacetamide-induced creatine kinase (CK) inhibition (27, 36) and CK knockout mice (25), muscle oxidative phosphorylation was activated more quickly than under control conditions when an active PCr system would normally buffer the increase in ADP. Glancy et al. (19) demonstrated that the time constant of O2 utilization by isolated mitochondrial preparations was related linearly to the total creatine (and PCr content) of the preparation. Also, Jones et al. (35) observed slower PCr kinetics when muscle PCr content was increased following a 5-day period of creatine loading, although pulmonary O2 uptake kinetics were not changed by a similar creatine loading regime (34a). Thus the lower baseline PCr content before Mod2 in the current study may contribute to faster muscle O2 utilization kinetics by shifting the CK reaction toward a higher ADP-to-ATP ratio during the exercise transition providing a greater drive for oxidative phosphorylation and resulting in faster VO2p kinetics.

In the present study, the baseline muscle ADPf content, while tending to be higher, was not significant, which may reflect the fact that the ADPf represents a calculated, whole muscle value, which may not directly reflect a mitochondrial ADPf concentration.

Muscle ADPf content was lower at end-exercise in Mod2 compared with Mod1, in spite of a higher end-exercise VO2p. The muscle ADPf content required to support a given steady-state rate of oxidative ATP synthesis is expected to be lower if provision of the other oxidative substrates is greater (28, 30, 56). As discussed above, provision of O2 and NADH may have been higher in Mod2, which may have reduced the requirement for a higher ADPf content to support oxidative phosphorylation. Thus the findings of elevated muscle PDH activity, NIRS-derived [Δ[HbTOT] and Δ[HbTOT]], and high muscle contents of ADPf and P1 prior to the onset of Mod2 compared with Mod1 suggest that the speeding of VO2p kinetics observed after heavy-intensity priming exercise in the older adults is a result of attenuating the delay in activating rate-limiting mitochondrial enzymes and providing substrate (reducing equivalents, ADP, P1) including O2 for mitochondrial oxidative phosphorylation.

Perspectives and Significance

This study has provided evidence that prior heavy-intensity exercise improves control of oxidative phosphorylation in older adults. This improvement would be expected to increase exercise tolerance, and thus, if applied appropriately, these findings may have practical implications for exercise prescription in older populations. In addition, we have demonstrated that this improvement in the control of oxidative phosphorylation in older adults appears to be the result of an integrative upregulation in the provision of all oxidative substrate (O2, elevated HbTOT and O2Hb; NADH, elevated PDH activity; ADP, lowered baseline PCR). Further work examining VO2p kinetics and exercise tolerance in older adults should therefore attempt to apply an integrative approach that considers the roles of all oxidative substrate.

In conclusion, this study demonstrated faster VO2p kinetics in older adults during the transition to moderate-intensity exercise when the transition was preceded by a bout of heavy-intensity exercise. Consistent with a greater contribution of oxidative phosphorylation during the transition to Mod2, there was a reduced net breakdown of PCr and a fall in muscle lactate content. An elevated PDH activity at baseline, which attenuated any delay in reaching the required steady-state PDH activity, and muscle content of ADPf and P1, and greater NIRS-derived [ΔO2[Hb]] and [Δ[HbTOT]] combined to attenuate any delay in delivering substrate for oxidative phosphorylation, and likely contributed to the faster adjustment of muscle O2 utilization and VO2p kinetics during Mod2. In addition, these results are consistent with the slowed VO2p kinetics typically seen in older adults as being the result of an impaired metabolic activation, in part related to the mitochondrial PDH complex (and/or possibly other oxidative rate-determining enzymes), in combination with impaired O2 availability.

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REFERENCES


25. Howlett RA, Hogan MC.


19. Gladden LB, Grassi B.


